UNITED STATES DEPARTMENT OF AGRICULTURE

BULLETIN No. 809

Contribution from the Bureau of Entomology
L. O. HOWARD, Chief

Washington, D. C. PROFESSIONAL PAPER March 10, 1920

# AMERICAN FOULBROOD

By

G. F. WHITE
Specialist in Insect Diseases

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## INTRODUCTION

American foulbrood is an infectious disease of the brood of bees caused by *Bacillus larvae*. The disease is characterized by a decided ropiness of the decaying brood and a peculiar foul odor. It is very widely distributed, is readily recognized, and is of much economic importance. Its existence has been known for a long time, and beekeepers have established many facts concerning it through observations made while practicing their profession. While there is a consensus of opinion among beekeepers on certain points regarding the disorder, there are others on which a diversity of views has existed and still others about which almost nothing of a definite nature has been known.

Former work was directed primarily toward the determination of the cause of the disease. Among the problems considered in the present studies are: The resistance of *Bacillus larvae* to heat, drying, sunlight, fermentation, and disinfectants; the effect of the disease on the colony and on the apiary; and the transmission, diagnosis, and prognosis of the disease. Direct studies on the treatment of the

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disease have not been attempted by the writer. It will be readily recognized, however, that any treatment that is efficient and at the same time economical must be determined by results obtained by the solution of such problems as those which have received attention in these studies.

The facts may tend to engender fear for the disorder in some instances while in others they may tend to allay it and to offer encouragement. It is hoped, however, that no statement made here will cause any beekeeper to lessen the vigilance that the disease requires, nor, on the other hand, to increase it to a point that would render its control uneconomical.

The discussions in the present paper are based almost entirely upon observations made in the laboratory and in the experimental apiary. The value of the results is emphasized by the fact that the disease produced experimentally and the disease encountered in nature are identical in almost every respect. It is believed that the paper[1] will be of interest not only to the practical beekeeper who wishes to apply the results noted here in the practice of his profession, but also to those who may desire to make further studies on the disease.

## NAME OF THE DISEASE

That bees suffer from diseases is recorded in works written before the Christian era but it is not altogether clear what the diseases were. In 1771, Schirach (19)[2] was using the term "foul brood" for an abnormal condition of the brood of the bees, but from his conception of the cause of the disorder one is led to believe that more than one abnormality was being referred to by the term. In 1882 Dzierzon (11) had definitely concluded that there were two kinds of foulbrood. Cheshire expressed a similar belief in August, 1884, but by September he had reached the conclusion that there was but one.

In 1885 Cheshire and Cheyne (9) published an article containing the results of some studies on foulbrood including a description of *Bacillus alvei*. For more than a decade after the appearance of the paper, the view was quite generally accepted that there was but one disease present in the condition that was being called foulbrood and that *B. alvei* was the cause of it. Many American beekeepers, those in New York State especially, became convinced, some time during the decade from 1890 to 1900, that two serious brood diseases were being referred to by the one name—foulbrood.

That there are two such diseases has been conclusively proved. In the United States the one characterized by a decided ropiness of

[1] The studies reported in the present paper are similar in nature to those made by the writer on sacbrood (25), Nosema-disease (26), and European foulbrood (27). These papers may be helpful where the discussions in the present one are especially brief. The investigations were completed in September, 1916, and the paper was submitted for publication in October, 1918.

[2] Figures in parenthesis refer to "Literature Cited," p. 42.

the decaying brood and a peculiar foul odor is now called American foulbrood, and the other one which is not so characterized is called European foulbrood. These two foulbroods are very different, the principal point of similarity being that they are both brood diseases. Both of them occur in Europe as well as in America. Unless these facts are borne in mind the names are likely to be misleading.

The term "foulbrood" (French, *la loque*) in most countries as in America frequently is used in a general sense meaning simply some disorder of bees but no definite disease. In this popular use of the term, either or both of the two foulbroods may be meant. Other brood disorders sometimes are loosely referred to by this general term. "Foulbrood" and "bee pest" or slight modifications of these terms as used in different countries certainly include the disease American foulbrood. In Switzerland no pronounced odor had been observed in connection with American foulbrood and it is referred to as "nichtstinkende Faulbrut" (6, 7, 8); in Austria it is called "Faulbrut" (18); in Germany "Brutpest" (29) and "Faulbrut" (12); in Denmark it is called "Bipest" (2, 3, 4); in England (10) and in Ireland it is called foulbrood or bee pest; and in Australia it is called foulbrood (5).

There are at least three infectious brood diseases of bees but as a rule the one which beekeepers, entomologists, and pathologists have referred to in the past by the term "foulbrood" is the disease American foulbrood, discussed in the present paper.

## HEALTHY BROOD AT THE AGE AT WHICH IT DIES OF AMERICAN FOULBROOD

The description of the symptoms of a brood disease as well as the recognition of the disease are very materially aided by making a comparison of diseased with healthy brood. Such a comparison involves the age of the brood, the relative arrangement of the capped and uncapped cells, the appearance of the diseased and of the dead brood, and the relation of the dead larva or pupa to the cell in which it lies. Furthermore, the character of the caps and the odor of the brood-combs should not be overlooked.

In healthy brood-combs a certain regularity is to be expected in the arrangement of areas containing eggs, larvæ, pupæ, and emerging brood, respectively. The cell caps when recently constructed are convex outward (Pl. II, A), but usually become flattened somewhat before the bees are ready to emerge. They are rarely punctured, remaining as a rule entire. In color these combs vary widely from a very light hue when recently constructed to a dark brown when old. Accompanying them is a slight but not disagreeable odor.

In American foulbrood the brood that dies does so nearly always either during the last two days of the four-day prepupal period or

the first two days of the pupal period. Such brood, therefore, is in capped cells. A description of a healthy larva at this age is given by the writer in a paper on sacbrood (25) and will be recounted here only briefly.

A larva (prepupa) (Pl. II, D, G; Pl. VI, G), at the age at which death from American foulbrood takes place as a rule, lies extended lengthwise in the cell with its dorsal side against the floor. It is motionless with its head in the direction of the mouth of the cell, its extreme anterior end extending nearly to the cap at the angle formed by the cap and the roof. Its posterior third lies upon the bottom of the cell and extends to the roof; its length is approximately that of the cell, being about one-half of an inch; its width is that of the cavity of the cell, about one-fifth of an inch; and its two lateral sides cover about one-half each of the two lateral walls of the cells. The ventral surface of the larva is in general convex from side to side and concave from end to end. Transverse ridges and furrows give a segmented appearance to the body. An empty space of considerable extent occurs between the ventral surface and the roof of the cell (Pl. VI, G).

The color of the larva at this time is almost white with a slight bluish tint. The entire surface is glistening. The body wall is sufficiently resistant to permit the removal of the larva intact if care is exercised. The tissue mass as seen when the body wall is ruptured is semiliquid and nearly white in appearance. Upon microscopic examination it is found to be made up very largely of fat cells, and to be free from bacteria and other microorganisms.

The change from the larva to the pupa (Pl. VI, B), as far as outward appearances are concerned, takes place rapidly. Infrequently death from the disease takes place during this short period. At this time the bee (Pl. VI, B) has neither the outward form of a larva nor of an adult. This stage of the transformation is recognized by the fact that the head is smaller than that of the complete pupa and the appendages are largely wanting.

The pupa (Pl. IV, A, D) immediately after transformation is similar in form and size to the adult bee. It rests with its back against the floor of the cell and with its antennæ, proboscis, and legs lying along its ventral surface. The posterior third is more pointed and much smaller than the same third of the larva. It lies upon the bottom of the cell but does not extend to the roof.

The color of the pupa at the time of its transformation is nearly white with a slightly bluish tint. The first pigment observed is seen in the compound eyes. Pigmentation of other parts of the body follows. When death from American foulbrood takes place, it does so almost invariably before the time that pigmentation of the body other than of the eyes has occurred. The body wall of

the healthy pupa for the first two days of this stage is easily ruptured. Its tissues are soft and when crushed are creamlike in consistency. During this period some care must be exercised in removing the body intact. Soon the body wall toughens so that the form is then maintained rather rigidly.

## SYMPTOMS

Much of our knowledge concerning the symptoms of American foulbrood has been gained through observations made by beekeepers while practicing their profession. The apiary in which the disease has been produced by experimental inoculations offers an opportunity to obtain a fairly complete picture of the disease. The present discussion of symptoms of the disorder is based upon observations made on the disease thus produced. During these studies it has been possible to duplicate observations already made by beekeepers on the disease as it occurs in nature and to make still others. It is quite probable that a number of these additional observations could be duplicated on the disease in nature if a sufficiently close study were made.

### GENERAL SYMPTOMS

In American foulbrood the symptoms vary within wide limits. The colony may or may not be noticeably weakened. If recently infected the strength will not be affected appreciably, but if the infection has been present for a considerable period the colony will be weakened as a rule. Not infrequently during the course of the disease the strength of the colony is diminished and death is the result. Between these limits any degree of strength may be encountered.

The occasional larva which dies of American foulbrood (Pl. VI, A) before it reaches the age at which healthy larvæ are capped rarely, if ever, is capped after its death. Aside from these few larvæ the brood that dies of American foulbrood does so in capped cells. After death the caps are removed by the adult bee from a large but varying proportion of cells containing dead brood (Pl. III, C, F, I; Pl. V, B), although they are allowed to remain on a considerable proportion of them (Pl. VI, C, E, F; Pl. II, B, C, E, F, H, I; Pl. III, A, B, D, E, G, H; Pl. IV, B, C, E, F; Pl. V, A, C, D, E, F). The irregular condition of the brood comb sometimes referred to by beekeepers as the "pepperbox" appearance (Pl. I, A, B, C) is due very largely to the presence of cells containing dead brood which have been uncapped by the bees, occurring among similar cells which have not been uncapped, together with cells containing healthy brood either capped or uncapped.

The caps over dead brood (Pl. II, B) appear in many instances similar to those covering healthy brood. In many instances,

however, they are altered, being sometimes punctured (Pl. III, A; Pl. VI, C), sometimes sunken, and sometimes darkened. Sometimes these abnormal appearances are combined. The holes in the caps vary in number and dimensions. Ordinarily there is but one (Pl. III, A), although there may be two (Pl. VI, C) or more. They are usually the size of a pinhead or smaller. The puncturing of the caps is done by the adult bees, and at the time of observation any proportion of the cap may have been removed.

Sunken caps are found only after the disease has been present in the colony for a considerable period, weeks at least. This symptom is most marked after the brood frames have been roughly handled, as in shaking bees from them or shipping them by express or mail (Pl. I, C). This condition of the caps is encountered more frequently in samples shipped to the laboratory than in those taken directly from the affected colony. By rough handling the viscid decaying mass within the cell is brought in contact with the cap, adheres to it and tends to draw the cap inward as it settles, accounting in a large measure for the condition referred to as sunken cappings. The dark caps occur somewhat later and also depend largely upon the presence of decaying brood material within the cell. The caps of many cells containing dead brood, however, are neither sunken nor darkened by its presence.

When recently dead the decaying brood in American foulbrood is light brown in color, the shade deepening as the process of decay continues. The color passes through a chocolate, coffee, and mahogany brown, reaching a very dark shade as it approaches the scale stage.

The body wall of a larva or pupa dead of the disease soon softens and is easily ruptured, making it impossible to remove the remains intact from the cell. As the process of decay continues the mass becomes viscid and before the scale stage is reached the viscidity is such that it is capable of being roped out into fine threads to a distance of 2 or 3 inches and sometimes even more. The scale when dry adheres more or less firmly to the cell wall.

The odor of the brood combs, when it is present, is characteristic of the disease and may be spoken of conveniently as the foulbrood odor. It is never detected in the early stages of the disease and if only scattering cells are present seldom is noticed. When much brood is dying and the disease has been present for weeks, the odor is noticeable and sooner or later becomes marked. When marked it is recognized by its strong and penetrating character and is usually thought of as being disagreeable. The brood combs after their removal from the hive tend to lose this foulbrood odor. The presence and extent of it in samples of the disease vary considerably, the variation depending largely upon the facts just mentioned.

It is possible to recognize most cases of American foulbrood from the general symptoms that are present. On the other hand many cases can be diagnosed only by an exact knowledge of the postmortem conditions of brood dead of the disease. A close study of these appearances, therefore, is advisable. The following somewhat brief description of larvæ and pupæ dead of the disease will suffice in most instances and will serve as a guide to further observations.

#### SYMPTOMS MANIFESTED BY THE BROOD

Signs that will determine the exact time of death from American foulbrood have not yet been ascertained. As the larvæ and pupæ that die of the disease do so at a period in their growth when healthy brood is motionless, lack of motion is no guide. In the descriptions made in the present paper it is assumed that a larva or pupa is dead if it shows a change from a bluish-white, more or less transparent appearance to one that is more nearly white, more nearly opaque, and shows at the same time a change from the normal turgidity to a slightly flaccid condition.

The appearance of the larval and pupal remains changes gradually from day to day from that of a healthy brood to that of the dried residue—the scale. A description, therefore, that would be correct for one day would be incorrect probably for the following day. Furthermore, all of these remains do not pass through the same changes. For convenience in description, therefore, the various appearances assumed by them are considered in five more or less arbitrary stages. The interpretation of the descriptions will be aided if the description of healthy brood (p. 3) is borne in mind, since the terms used are similar in both instances.

#### LARVÆ DEAD OF AMERICAN FOULBROOD

The descriptions made of dead larvæ (prepupæ) for the most part will be of those that have died during the 2-day quiescent period just preceding the transformation to the pupa, as most larvæ dying of the disease succumb at this age.

##### FIRST STAGE

The first symptoms of American foulbrood appear about the end of the first week after infection. From the bluish white of the healthy larva the color changes during this first stage of the disease to a very light brown. The conelike anterior third (Pl. II, E) having settled somewhat, the apex is now slightly farther from the roof. The surface markings in each of the three thirds (Pl. II, H) are very similar to those of a healthy larva. The body wall is easily ruptured, but by care the larva may still be removed intact from the cell. In consistency the decaying tissues are soft and nonviscid.

Sometimes a considerable portion of a larva is removed piecemeal by the bees during the first stage of decay. The remnant (Pl. VI, D), consisting of the posterior third and usually much of the middle third, is found occupying its normal position in the cell. The surface markings are similar to those of the middle and posterior thirds in the first stage of decay. The surface left by the removal of the fragments is somewhat roughened and transverse to the body. These larval remnants are similar to those seen in sacbrood, but in American foulbrood there are fewer of them. When present they form one of the earliest symptoms of the disease.

SECOND STAGE

By the second week after death the larva shows a slightly deeper shade of brown, although still quite light. The anterior third (Pl. II, F) is somewhat darker than the other two thirds. The apex is farther from the roof than in the first stage. The surface markings (Pl. II, I) are now less pronounced throughout. An attempt made at this time to remove the dead larva from the cell results usually in rupturing the body wall. The decaying mass can be entirely removed, however. The consistency of the tissue mass in this stage is somewhat similar to that of moist dough. It has not yet reached the true viscid condition.

THIRD STAGE

By the third week after death the remains have assumed a medium shade of brown, approximating that usually seen in brood cappings. The apex of the anterior third (Pl. III, D) is now widely separated from the roof of the cell, exposing to view the ventral surface of the remains. The transverse ridges and furrows marking the segments (Pl. III, G) are practically obliterated. The edges no longer show the deep notches. The side-to-side convexity is decreased. Evidences of developing head and thoracic appendages are sometimes seen. Transverse tracheæ can usually be observed as white lines across the ventral surface of the abdomen, one in each segment. The body wall is ruptured easily. The decaying mass shows some viscidity and adheres to the walls of the cell.

FOURTH STAGE

By the fourth week after death the color of the dead larva reaches a deep brown, being in shade similar to that of the average older brood combs. The apex of the anterior third (Pl. III, E) is raised only slightly above the upper surface of the decaying larval mass. After being uncapped this third is the first to dry, becoming dark and scale-like. Surface markings practically have disappeared; tracheæ frequently are still visible, especially in the middle third. The ventral

surface (Pl. III, H) is now slightly concave from side to side. The posterior third lies upon the bottom of the cell and extends to the roof. The decaying larval mass is now decidedly viscid in consistency. When tested with a toothpick, match, forceps, or any other suitable object it can be drawn out into threadlike strings. This viscid condition of decaying brood is referred to by beekeepers as "ropiness." It is a well known symptom and is much used in the diagnosis of the disease.

#### FIFTH STAGE

One month or longer after the death of the larva the remains are found to be a thin mass more or less dry and covering most of the floor, some of the side walls, and the bottom of the cell. The dried mass is known to beekeepers as the "scale." Its color is dark brown, similar to that of old brood comb. That portion which was once the anterior third of the larva bears, if at all, only a slight elevation (Pl. III, C, F, I; Pl. VI, E, H). The ventral surface (Pl. III, I) is concave from side to side and quite uniform. The posterior third covers the bottom of the cell and extends to the roof. This can be seen especially well by cutting lengthwise the cell with a scale (Pl. VI, H) in it. It can be seen also that the thickness of a scale throughout the median line is approximately uniform.

As some larvæ die of American foulbrood before the quiescent stage is reached but after being capped, their dead remains may be found occupying not the uniform position just described but various positions in the cell. While the form of the remains, therefore, may vary materially, the color and consistency pass through stages that are similar in all instances.

Occasionally death from American foulbrood takes place while the larva is still younger, i. e., before the time of capping has arrived and while it (Pl. VI, A) is coiled in the cell. This condition is comparatively rare. As the form of the remains depends upon the age of the larva at the time of its death, naturally, the form of the remains of a larva dying before the time for capping will vary materially from the descriptions above. The color and consistency of such a larva during its decay, however, pass through stages that are similar to those already described.

### PUPÆ DEAD OF AMERICAN FOULBROOD

Death from American foulbrood does not take place late in the pupal stage but almost always, if not invariably, within the first 2 days after transformation from the larva (prepupa). At this stage the healthy pupæ are practically white, with or without pigment in the compound eyes. Pupæ dead of the disease resemble in color and consistency larvæ dead of the disease. The dead pupæ, as in the case of the larvæ, are described here in five stages.

FIRST STAGE

During the second week following the infection, symptoms of American foulbrood may be seen in pupæ. The cell containing a dead pupa is generally found capped at this time. Upon uncapping, the anterior third (Pl. IV, B) of the pupa will be recognized as resembling very closely that of a healthy one. The surface markings in general (Pl. IV, E) are not particularly unlike those of a healthy pupa. The turgidity is slightly less and the body is to a slight degree more nearly opaque. The body wall is easily ruptured and the tissue mass is soft but not viscid. In general the consistency is similar to that of a larva in the first stage of decay.

SECOND STAGE

The process of decay continuing, the tissues soften and the pupal mass settles, distorting its form. The anterior third (Pl. IV, C) has settled to the floor of the cell and is separated from the roof by a considerable distance. The face is directed more nearly upward. The surface markings (Pl. IV, F) of each third are less distinct. The legs and other appendages rest upon the body. The body wall is at this time very easily ruptured and the tissues are soft. The color and consistency are similar to those of the second stage of decay of the larva.

THIRD STAGE

During the third stage the appendages are less easily distinguished; as they settle they merge more or less with the decaying mass of the head, thorax, and abdomen. One exception should be mentioned. Some of the mouthparts (Pl. V, A), chiefly the proboscis, adhere to the roof of the cell. This condition has often been noted by the beekeepers. The face of the pupa in this stage is directed still more nearly upward than in the preceding stage. The head is not infrequently found to be disfigured through drying or being gnawed or both. The ventral surface of the middle third (Pl. V, D) is still, in general, convex from side to side. The color and consistency are similar to those of the same stage in the larvæ. When the pupal mass is removed white lines may be seen in it. These are tracheæ.

FOURTH STAGE

In the fourth stage evidence of drying is marked and pupal remains are changed still further. The anterior third (Pl. V, B) bears only a slight resemblance to that of the pupa. Through settling of the mass and through drying it is now very much flattened. The ventral surface of the middle and posterior thirds (Pl. V, E) are still slightly convex from side to side and are roughened, due chiefly to the remains of the legs and other appendages. The color of the decaying mass in the fourth stage is a dark brown similar to that

of old brood comb. This mahogany-hued mass is viscid in consistency, showing the ropiness that characterizes brood dead of American foulbrood.

#### FIFTH STAGE

Finally after a few weeks of decay and drying there is to be found in the cell the more or less dry residue of the dead pupa—the scale (Pl. V, C, F; Pl. VI, F, I). It covers most of the floor and some of the side walls and only a portion of the bottom. The pupal scale is in many respects similar to the larval scale. It is concave from side to side. The ventral surface is slightly roughened by the dry decayed appendages. The posterior third extends only slightly upon the bottom of the cell. This can be demonstrated by cutting the scale (Pl. VI, I) lengthwise as it occupies its position in the cell. The scale is dark brown resembling in shade that of old brood comb. It adheres rather firmly to the cell but with care can be removed from it. When thoroughly dry and removed it is found to be quite brittle.

## ETIOLOGY

### PREDISPOSING CAUSES

*Age.*—American foulbrood infection takes place only during the feeding stage of larvæ. Death occurs almost invariably after the feeding stage is passed, i. e., after capping, and either while in the larval stage or soon after transformation to the pupa. Older pupæ do not die as a result of the disease and adult bees do not become infected.

*Sex.*—That worker, drone, and queen larvæ are all susceptible to the disease has been demonstrated during these studies. Affected drone brood is encountered less often in the diagnosis of this disease than in that of European foulbrood. The writer has encountered queen larvæ affected by American foulbrood in experimental colonies only, although very probably diseased queen larvæ do occur in nature also.

*Race.*—Thus far no race of bees has been shown to possess complete immunity from the disease. In the experimental inoculations recorded in the present paper bees mixed with Italian blood were used for the most part. The queens in many of the colonies were purchased as "untested Italians." At least five colonies of "tested Italians," two of "tested Carniolans," and two "tested Caucasians" were inoculated. Among the colonies used there were also several common black bees. The disease was readily produced in all of these strains. Furthermore, the results obtained from the examination of numerous samples of diseased brood received from beekeepers throughout the United States indicate that all strains of bees commonly found in American apiaries are susceptible to infection with American foulbrood. No definite conclusion can be drawn at the present time regarding the relative immunity possessed by the different races.

*Climate.*—It is quite well known that American foulbrood is very widely distributed. The writer has examined samples of the disease from England, France, Germany, Switzerland, New Zealand, Canada, Cuba, and various parts of the United States. This is sufficient to show that the disease exists under a great variety of climatic conditions. The practical import of the observation is that the presence of American foulbrood in any particular locality can not be attributed entirely to the climatic conditions of the region.

*Season.*—In general the losses from American foulbrood occur later in the bee season than do losses from either sacbrood or European foulbrood. Experimental inoculations have shown that the larvæ of bees are susceptible at all seasons and that the disease can be produced whenever brood is being reared. It would seem, therefore, that the severity of the disease is due more to environmental conditions existing at the different seasons than to any difference in the susceptibility of the larvæ during these periods.

*Food.*—Since American foulbrood occurs in such widely different localities (see "*Climate*"), wherein the food of bees varies almost as much as it is possible for it to vary, it may be concluded that the quality of the food used by bees has very little, if anything, to do in the causation of the disease. Furthermore, it is found experimentally that the disease can be produced when the colony is well supplied with food, when there is a moderate quantity present, or when there is a scarcity. It would seem from the facts at hand that the course of the disease probably is governed to some extent indirectly by the quantity of food present and to a less degree, if at all, by its quality. The value of these factors has not been determined but it is certainly not great in either case.

### EXCITING CAUSE

During the period from 1885 to 1902 (p. 2), *Bacillus alvei* was assumed quite generally to be the exciting cause of foulbrood. From 1902 to 1907 much interest was manifested in the problem relating to the cause of the foulbroods. This is shown by the investigations of Lambotte (13) in Belgium, Burri (6, 7) in Switzerland, Bahr (2) in Denmark, Maassen (14) and Erne (12) in Germany, and those of the writer.

The writer's experience with the bee diseases began in 1902 when he was working under the direction of Dr. V. A. Moore. In the first studies made, spores were found in very large numbers in the scales of the ropy foulbrood. As these did not grow on the media commonly used in a laboratory (17) it was at once recognized that they were not the spores of *Bacillus alvei*. Furthermore, as *B. alvei* was not found in any of the samples of the ropy disease, the conclusion was drawn very naturally that this bacillus could not possibly be its cause.

*Bacillus alvei* was found to be present, however, with much regularity and in great numbers in the brood disease that is not charac-

terized by ropiness and foul odor—European foulbrood. These observations taught, therefore, that *B. alvei* was not to be associated with American foulbrood—the ropy, foul-smelling disease—but with European foulbrood, which does not possess these characteristics.

After beekeepers had convinced themselves that there were two foulbroods, one with a peculiar foul odor and showing a marked ropiness of the dead brood and the other without these characters, *B. alvei* unfortunately remained in the literature as the cause of the ropy, foul-smelling disease. This fact accounts for not a little of the confusion that has existed in connection with the brood diseases. It should be remembered that *B. alvei* is not the exciting cause of any bee disease.

The writer found (20) that the spores which had refused to germinate on the media ordinarily used in the laboratory would germinate and the bacillus would grow in an agar medium in the preparation of which bee larvæ were used. Satisfactory cultures for experimental purposes were not obtained with this medium, however. Later he (23) succeeded in devising a medium (p. 18) by the use of which pure cultures of *Bacillus larvae* suitable for experimental work could be obtained in abundance and with such cultures American foulbrood was produced by inoculation, the experimentally produced disease manifesting symptoms that are typical of American foulbrood encountered in nature.

### BACILLUS LARVAE

*Bacillus larvae*, the cause of American foulbrood, had been seen by microscopists doubtless long before it was cultivated successfully in the laboratory. The species requires special media for its cultivation. Success in the germination of its spores was attained in 1903 on an agar made from bee larvæ. Pending more definite information regarding the bacillus the writer (20) referred to it by the term, Bacillus "X." Further knowledge concerning the species was gained during the summer of 1904, and it was given the name *Bacillus larvae* (White 21, 22). Burri (6) in Switzerland, working on the disease entirely independently, also recognized the fact that the spores present in such large numbers in the scales represented a new species that was difficult of cultivation. Maassen (14) has referred to the species as *Bacillus brandenburgiensis*, and Cowan (10) has referred to it as *Bacillus burrii* (8).

*Occurrence.*—*Bacillus larvae* occurs in the brood of bees dead of American foulbrood and where contaminations with such material have taken place.

*Morphology.*—The vegetative form is a slender rod with ends slightly rounded and with a tendency to grow in chains (fig. 1; Pl. VII, A). It varies markedly in length, depending largely upon the medium used in its cultivation. On the surface of brood-filtrate agar it is more often from 2.5 to 5 $\mu$ in length and about 0.5 $\mu$ in breadth. In a liquid medium it is usually much longer, becoming then frequently filamentous in character. During the vegetative stage the bacillus undergoes changes (fig. 5; Pl. VII, E, F, G, H) seldom noted for the bacteria. The rod possesses numerous flagella which are peritrichic in arrangement (fig. 2; Pl. VII, C).

*Giant whips.*—Giant whips occur (fig. 6; Pl. VII, F, G, H) in large numbers, being found especially numerous in the water of condensation of brood-filtrate agar cultures. These corkscrewlike structures vary widely in their dimensions from scarcely visible coiled filaments to bodies several micra in diameter.

*Motility.*—The bacillus is moderately motile in young cultures taken from the surface of brood-filtrate agar but is somewhat more sluggish when grown in liquid cultures.

*Spore formation.*—On brood-filtrate agar evidence of spore formation is present about the third day. The rod is swollen near the center where the spore is formed, being then spindle-shaped (fig. 3; Pl. VII, B). In a few days numerous spores free

FIG. 1.—*Bacillus larvae:* Vegetative form.

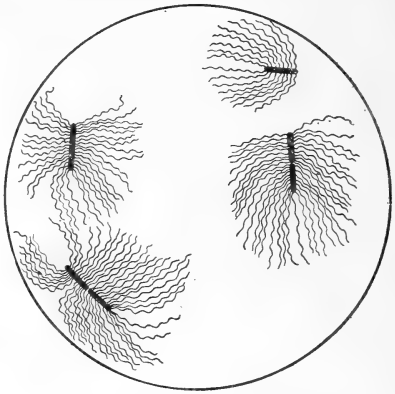

FIG. 2.—*Bacillus larvae:* The flagella.

from the rods are to be seen (fig. 4; Pl. VII, D). They measure about 0.6 by 1.3 $\mu$. In some environments few or no spores are produced. This occurs in liquid media, deep in solid media, and on media containing glycerin, mannite, or glucose. Some of the other sugars and also honey inhibit spore formation.

*Staining properties.*—The rods color readily and uniformly with the analine stains, and are Gram-positive. The spores are quite resistant to stains.

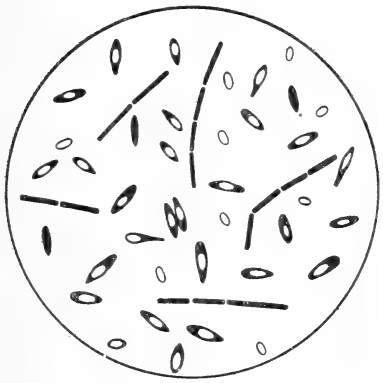

FIG. 3.—*Bacillus larvae:* Spore formation.

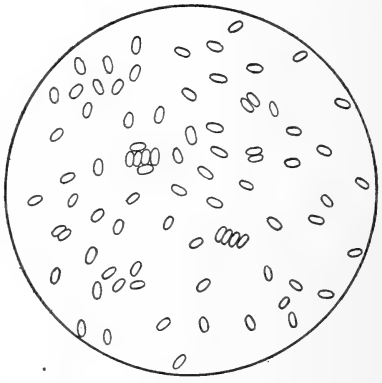

FIG. 4.—*Bacillus larvae:* The spores.

*Oxygen requirements.*—When bee larvæ agar alone is employed and inoculations are made with spores following Liborius' method for anaerobes, growth as a rule has appeared more often near to than on the surface (Pl. VIII, E, F, G, H, I). Subcultures on brood-filtrate agar (Pl. VIII, D) and egg-yolk-suspension agar or their combination yield abundant surface growth.

*Agar slant.*—On the surface of inclined brood-filtrate agar subcultures (Pl. VIII, D) grow rapidly, producing a moderate to heavy growth in 24 hours. This growth tends

to spread somewhat from the area inoculated, is grayish-white, and slightly viscid. It presents a more or less uniform border, a smooth surface, and a ground-glass appearance. Older cultures are less prominent than the younger ones.

*Agar plates.*—Colonies on the surface of agar vary in size depending upon the number present. When only a few are present not infrequently they spread and attain a diameter of a centimeter or more. The border is clearly defined and uniform. The growth is only slightly raised and has a smooth surface and a ground-glass appearance (Pl. VIII, A, B). Deep colonies vary from lenticular to irregular in form with filamentous outgrowths from portions of their surface (Pl. VIII, A, C).

*Fermentation.*—Carbohydrate liquid media as ordinarily prepared are not suitable for the growth of *Bacillus larvae*. In some of these after a considerable period a slight growth may appear at the bottom of the tubes. A little brood-filtrate or egg-suspension

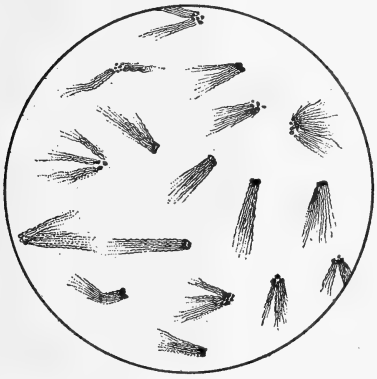

FIG. 5.—*Bacillus larvae*, illustrating an interesting feature of the organism.

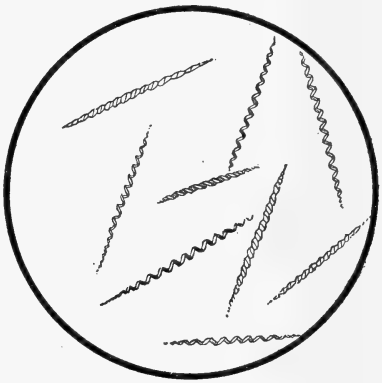

FIG. 6.—Giant whips from cultures of *Bacillus larvae*.

added to the media improves it. No visible gas is formed but in some instances slight acidity is produced.

*Gelatin.*—No growth takes place in plain or in brood-filtrate gelatin at temperatures at which it remains congealed.

*Power to resist disinfectants.*—The spores of *Bacillus larvae* are very resistant to heat. When suspended in water the more resistant ones require as much as 100° C. maintained for 11 minutes to destroy them and when suspended in honey require a half hour or more (p. 22). They resist respectively 5 per cent carbolic acid for months; 1 to 1,000 mercuric chlorid for days; 10 per cent formalin for hours; and 20 per cent formalin for minutes; each acting at room temperature.

*Pathogenesis.*—*Bacillus larvae* is pathogenic for the brood of honeybees. Infection takes place during the feeding period of the larvæ by way of the alimentary tract. The brood dies of the disease in the larval, prepupal or early pupal stages. The earliest symptoms to be observed following inoculation usually occur in prepupæ during the seventh day after the feeding, rarely earlier. The earliest evidence of infection in pupæ occurs one or two days later. The bacilli are at this time distributed throughout the body. Adult bees, rabbits, guinea pigs, and rats are not susceptible to infection with the parasite.

While the dead brood in the scale stage and in other later stages of decay in American foulbrood invariably contains spores of *Bacillus larvae* in immense numbers, a microscopic examination of earlier stages shows not only few spores but also a comparatively small

number of rods. A partial explanation at least of this rather unexpected fact was gathered from the study of the organism on artificial media. Here it was found that the rods at times undergo interesting but as yet undetermined changes. On glucose and other media, when the growth is luxuriant and the spore formation is very much inhibited, it is noted that in older cultures rods are comparatively few in number but in their stead are forms which are neither rods nor spores but are small, more or less spherical bodies (Pl. VII, F, G, H). The exact transformation that has taken place in the bacillus to produce these bodies is not known. Something of the phenomenon has been learned through a study of the rods stained with a flagellar stain employing in the method a light aqueous suspension of a young culture. In such preparations there is seen within the rods a number of individual elements, apparently, each of which is supplied with flagella. These structures become independent and separate from the rod proper (fig. 5; Pl. VII, E, F, G, H). In the cultures are found also, especially in the condensation water of brood-filtrate-agar slant cultures (Pl. VIII, D), giant whips (fig. 6; Pl. VII, F, G, H) of various sizes. That the little-understood changes that take place in the vegetative forms bear a close relation to the formation of the giant whips can readily be suspected.

Definite data have been sought to prove the identity of the ropy foulbrood of the different countries. Samples of brood comb containing disease material were received from Canada, Cuba, England, France, Germany, New Zealand, and Switzerland. The findings from studies made on these were compared each with the other and with the findings from many samples from the United States. Spores of *Bacillus larvae* in very large numbers and practically in pure cultures were present in every one. In each instance one of the special media (p. 18, 19) required for the cultivation of this species was needed. Culturally the bacillus was the same from all of the samples. The disease produced by the inoculation of colonies of bees was also similar in every instance. Sera from rabbits immunized with cultures from American sources agglutinated in high dilutions cultures from English, New Zealand, and Switzerland sources at least, these being all that were tested in this way. The evidence, therefore, justifies the conclusion that the ropy foulbrood of each of the different countries mentioned above is identically the same disease.

Four rabbits inoculated with pure cultures of *Bacillus larvae* showed comparatively little reaction. In each instance there was used a moderately clouded suspension in normal salt solution made from the surface of the brood-filtrate-agar slant culture about 24 hours old. Two of them were inoculated subcutaneously with 1 and 2 c. c. of the suspension, respectively; one received the culture intraperi-

toneally and one intravenously. The temperature taken of the latter two throughout the day of the inoculation was found to be increased about 3° F. following the inoculation. The next day they were somewhat sluggish with impaired appetite. The temperature, however, soon declined to normal and their feeding became normal. The 1,500 gram rabbits were from 100 to 200 grams light in weight on the day after the inoculation but soon the loss was regained. Observations were made over a period of 2 months. Autopsies on the chloroformed animals showed no abnormalities worthy of note.

Four guinea pigs inoculated subcutaneously with cultures similar to those used in the rabbit inoculations showed only slight reaction. The temperature of the one taken throughout the day of inoculation showed a rise of about 2° F. but was normal thereafter. No loss in the weight of these 400 to 500 gram guinea pigs was appreciable. One died in 10 days, apparently from causes foreign to the inoculation. The remaining three were chloroformed after 6 weeks and presented no abnormalities of note at autopsy.

Two gray rats were inoculated subcutaneously, one with the vegetative and the other with the spore form of *Bacillus larvae*. These proved to be refractory. After 4 weeks they were chloroformed and at autopsy no abnormality of interest was noted except a slight infiltration at the point of inoculation in the animal receiving the vegetative culture, and a small abscess in the case of the animal receiving the spore suspension. In the abscess spores were present.

## TECHNIQUE

Much of the time devoted to the study of American foulbrood has been consumed in the study of technique. Special media were required and the method of conducting the experimental studies had to be developed.

### MEDIA

Failure to recognize the fact that the spores occurring in such large numbers in the brood dead of American foulbrood do not grow on the media ordinarily used in the laboratory has caused a number of workers to go astray and has contributed not a little to the confusion that has existed concerning the brood diseases. Lambotte (13) in Belgium experienced some difficulty in obtaining cultures following inoculations with the spore-bearing disease material. He made a medium using the brood of bees in its preparation and with it obtained a growth which he interpreted as being the species represented by the numerous spores present in the brood dead of the disease. The interpretation was evidently incorrect. The culture which he obtained he identified as a member of the *mesentericus* group. In 1903, before learning of Lambotte's work, the writer (20) had made an agar using bee larvæ and obtained a germination of the spores of the

ropy foulbrood. In this bee-larvæ agar[1] the growth is always slow and somewhat feeble. Spores are produced to a slight extent only or not at all. Such cultures are unsuitable for experimental purposes.

A medium containing a sterile filtrate obtained from the brood of bees was devised by the writer (23) which meets the requirements of experimental studies. It is prepared as follows: Healthy larvæ taken from the brood comb are crushed and the crushed mass is diluted with water to several times its volume. This is placed in a flask, and after adding a few c.c. of chloroform the flask is stoppered and allowed to remain at incubator temperature overnight. The suspension then can be

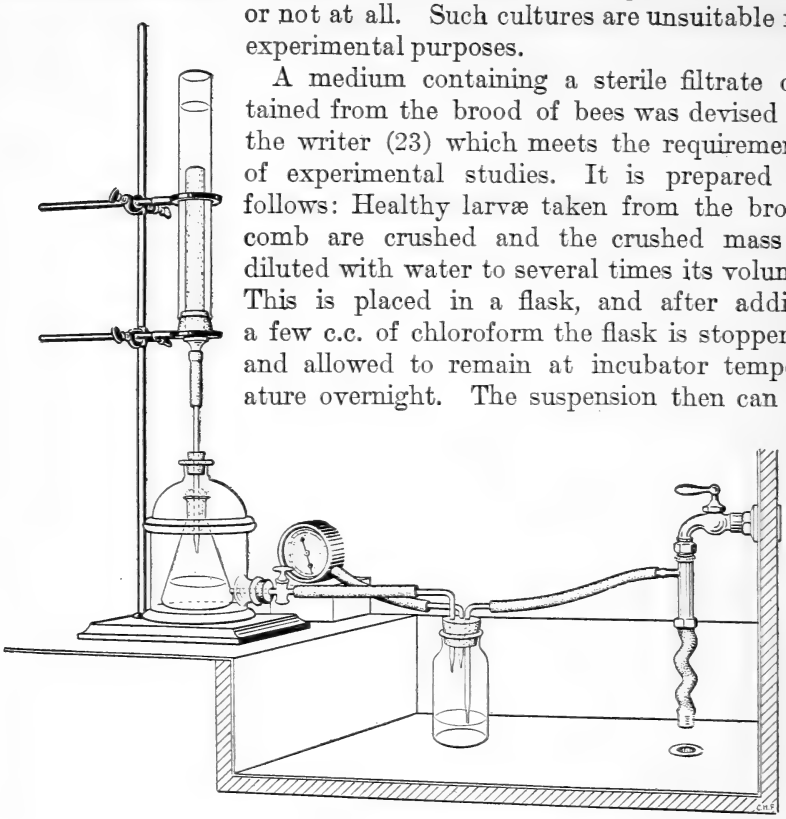

FIG. 7.—A convenient method for obtaining sterile filtrates.

filtered easily by using any bacteria-proof filter[2] (fig. 7). The filtrate is pipetted aseptically into sterile tubes and stored until

[1] Larvæ, prepupæ, or pupæ recently transformed furnish satisfactory brood material for making the bee-larvæ media. These are picked from the brood combs, crushed, and used as meat in formulas ordinarily followed in making bouillon and agar. Excess heating should be avoided. In making the inoculations an aqueous suspension of the spore-containing material heated to 100° C. for one or two minutes should be used. A tube of the special agar liquefied is inoculated with a loopful of the heated suspension. After cooling it is incubated at about 38° C. Three days or more may be required to produce a visible growth (Pl. VIII, E, F, G, H, I).

[2] In caring for and using the filter cylinders in these studies the following course has been pursued: The cylinder is immersed in water for a few hours or over night and is then sterilized in the autoclave. After being used for filtering a brood suspension it is washed in water without scrubbing. To remove further the brood material water is drawn through the cylinder under pressure as in filtering. As a rule it is again immersed in water and allowed to remain over night, after which, the filter being assembled, water is filtered again. It is then ready for use and may be stored until needed. Cylinders which have been used repeatedly in this way have lost none of their efficiency. The vacuum chamber is made in two sections similar to a Novy jar. Vaseline is used on the wide ground flanges. No rubber band or clamps are needed.

The flasks used in the vacuum chamber of the apparatus before being sterilized are stoppered with cotton wrapped about a small-sized test tube. The cylinder having been immersed in water is sterilized together with the rubber connections and glass tubing in place, but without the glass mantle. In assembling the apparatus sterilized connection between the cylinder and flask in the vacuum chamber is readily obtained through the opening in the plug left by removing the test tube. After a vacuum has been established in the chamber the stopcock may be closed and the pump turned off.

needed. About 1 c.c. of the brood filtrate is added to each 5 c.c. of agar liquefied and cooled to about 50° C. After being inclined the medium is ready for use and may be stored. Plating for pure cultures may be done by adding the brood filtrate to the agar at the time the plates are made.[1]

When it is desired to obtain cultures from the spore form of *Bacillus larvae,* brood-filtrate agar is not a suitable medium in itself. The spores should be germinated first by inoculating bee-larvæ agar (p. 18) or egg-yolk-suspension agar. By plating in 2 or 3 days the vegetative form thus obtained, using brood-filtrate agar, pure cultures can be assured. Studies have shown, however, that *Bacillus larvae* is present in practically pure cultures in brood dead of the disease, and by heating the spore-containing material in aqueous suspension at 100° C. for one or two minutes as suggested above the occasional contaminating organisms are eliminated usually without plating.

Bee-larvæ agar is limited in its usefulness on account of the large amount of brood required in its preparation. A more suitable medium, therefore, was sought. An unheated egg-yolk agar was found by the writer (28) to be very satisfactory. This is prepared as follows: Fresh eggs are used. After having been immersed in a disinfecting solution the shell is broken, the white of the egg is poured off, and the yolk is dropped into a flask containing about 70 c.c. of sterile water. With a sterile pipette the aqueous suspension of yolk, resulting from agitating the flask, is transferred to sterile tubes and stored until needed. In making an egg-yolk-suspension agar about 1 c.c. of the egg-yolk suspension is added to each 5 c.c. of agar in tubes liquefied and cooled to about 50° C. The agar is inclined and may be stored until needed.[2]

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[1] Larvæ, prepupæ, or young pupæ may be used in obtaining the brood filtrate. The use of sterile water is a good precaution in making the dilution of the crushed brood mass. A dilution of 1 to 10 up to 1 to 50 gives very satisfactory results, although lower and higher dilutions have been used with success, while at incubator temperature an autodigestion apparenty takes place. The "autodigested" suspension is passed through filter paper before the bacteria-proof filter is used. For the latter the Pasteur-Chamberland F is very satisfactory. (Fig. 7.) The B grade has been used but the filtering is slower. The Berkefeld N has also been employed, but is less efficient. In using this latter type of filter gravity alone should be employed. If the weather is warm, or if the filtering is not to be done for a few days, the aqueous suspension may be allowed to remain at room temperature. This permits the changes which take place to advance sufficiently to make the filtering process comparatively easy. The chloroform saturated suspension of brood material has been kept at room temperature for more than three years without its usefulness being impaired to any appreciable extent. After a considerable period the suspension becomes practically sterile and has been used without being filtered. When an early use of the filtrate is desired its sterility should be tested by placing the tubes containing it at incubator temperature for a few days. Sometimes it is desirable to use other brood-filtrate media than the agar. In this event a small amount of brood-filtrate may be added to any one of the media ordinarily used in the laboratory. Such special media usually support a growth of *Bacillus larvae* when inoculated with the vegetative form of the organism.

[2] Eggs obtained from the market labeled "strictly fresh" have been suitable as a rule for the egg-yolk suspension. Eggs known to have been recently produced are to be preferred, however. Almost any of the more common efficient disinfecting solutions may be employed for sterilizing the eggshell. Aqueous solution of mercuric chlorid 1:1000; carbolic acid 5 per cent; formalin 10 per cent; and alcohol each have been used, mercuric chlorid being preferred. To insure sterility autoclaving of the flasks with the contained water has been practiced. A wide-mouthed flask is preferred. In securing the yolk aseptically, it

### EXPERIMENTAL INOCULATIONS

A very satisfactory colony for inoculation purposes is a queen-right nucleus that can be accommodated comfortably on from 3 to 5 brood frames approximating the Langstroth size. The hive, the arrangement of the apiary, the colony, the feeding, and the manipulations in general are similar in the experimental study of American foulbrood to those employed by the writer in the study of sacbrood (25), Nosema-disease (26), and European foulbrood (27). The inoculated colonies were, therefore, in the open. Precautions tending to minimize the likelihood of robbing, swarming, absconding, and the accidental straying or drifting of bees should not be overlooked.

The inoculations are made by feeding a suspension of the spores of *Bacillus larvae* in sirup or honey, the spores being obtained from pure cultures or from brood dead of American foulbrood. Three or four tubes of spore-containing cultures grown on brood-filtrate or egg-yolk-suspension agar furnish a suitable quantity of the virus. Likewise the spores contained in three or four scales give satisfactory results. In some experiments, feedings made on successive days are desirable. Direct inoculation by means of a capillary pipette is less satisfactory in experiments on American foulbrood than in those on sacbrood.[1]

The first symptoms of American foulbrood to be observed following the inoculation usually appear in the worker larvæ by the end of the first week and not earlier than the sixth day. The affected larvæ are in capped cells. Not infrequently the first evidence of disease noticed is the remains in cells here and there of partially removed

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is convenient to break the sterilized shell about one end of the egg, remove the pieces with flamed forceps, and, after making a small hole in the other end, pour off the white. The yolk remaining is poured into the flask. By breaking the limiting membrane of the yolk at the time of pouring into the flask the process is facilitated. Different degrees of dilution of the egg yolk have been employed, 60 c.c. to 80 c.c. of water giving satisfactory results. Any ingredient, such as the sugars, desired in the final medium may be added to the water of the flasks. The egg of the hen has been used in most of the studies reported here. Eggs of the duck were also found to be suitable. Occasionally a contamination of the egg suspension will be found. In these cases the suspension becomes firm after incubation resembling then the consistency of coagulated milk cultures. If the contamination is present it will be apparent in a few days after storing, or it may be determined earlier at incubator temperature. The sterile egg-yolk suspension as an ingredient for special media retains its efficiency for a long period. Indeed an aqueous suspension of the dry residue from the yolk supension was found to be efficient after 3 years, although its value is then apparently somewhat impaired.

Maassen (15) used bee-larvæ and also brain agar in studying *Bacillus larvae*. The egg-yolk suspension agar, in the writer's experience, has advantages over the brain agar.

[1] Cultures on the surface of agar are readily suspended in water as is also the disease material in the decaying larvæ and scales of American foulbrood. These aqueous suspensions are used in making the sirup or honey suspensions. From 1 to 3 c.c. of water for each scale is a convenient proportion. Sirup is made by bringing to the boiling point an aqueous suspension of granulated sugar in which the sugar used exceeds that of the water. When honey is used for the spore suspension it is diluted with water, the amount of water added being slightly less than that of honey. In most instances honey is less suitable than sirup inasmuch as it tends to encourage robbing especially during a dearth of nectar, it needs to be sterilized before use, and is more expensive. It was found that less than one scale is sufficient disease material to produce a considerable amount of disease in the colony. In some experiments one scale, therefore, might supply all of the spores needed although the use of a somewhat greater quantity of material is advisable in most instances.

larvæ (Pl. VI, D), about one-third of the larva being removed. The transparency seen in healthy larvæ disappears, and the bodies of the ones affected become more nearly opaque. Soon the color assumes a tint of brown which deepens as the process of decay continues, passing through chocolate, coffee, and mahogany shades. In the earliest stages of the disease the body wall is ruptured more easily and the tissues are softer than in healthy brood. As the disease advances a ropiness of the decaying brood is to be observed, the characteristic odor appears, and a loss in the strength of the colony is evident. After a time an irregularity in the appearance of the brood comb is to be seen, the capped and uncapped brood being abnormally distributed with here and there perforated and sometimes sunken caps (Pl. II, A, B, C). Still later the scales are found.

The time at which the various symptoms appear varies. Climatic conditions, the amount of infection present, and the initial strength of the colony are some of the more important factors causing the variations.

Whether a hive which has housed a colony infected with American foulbrood will transmit the disease is a question which has not been altogether solved but in experimental work all hives that have housed such colonies should be disinfected before they are used again. This can be done satisfactorily by flaming the inside of the hive. If gas is available the Bunsen burner is very satisfactory for this purpose. That no fear need be entertained from hives which have been flamed properly is demonstrated by the fact that the disease has not been transmitted by flamed hives in the experiments made in the present studies, although the number of such hives which have been used is large. The length of time that a sirup or honey suspension of American foulbrood spores stands before the inoculation of a colony is made need not be considered in experimental work.

From what is known of American foulbrood its transmission by queens is not to be expected. Two queens from diseased colonies were introduced into healthy ones in October and the colonies were kept under observation until the following July. The disease did not appear in either of them. In a number of instances during the investigations queens from American foulbrood colonies were used to queen nuclei made by division of healthy colonies. These colonies were under observation for weeks or months before they were used for American foulbrood experiments. In no instance was the disease observed to result from the use of these queens. The conclusion is reached, therefore, that for most experimental work at least the possible previous relation of the queen to diseased colonies need not concern one.

The combs from an American foulbrood colony never should be given to a colony which is to be used for experimental purposes.

The bees from diseased colonies may be used again in certain well-selected cases by transferring them to another hive which may be supplied with comb from healthy colonies or with foundation strips or full sheets. To insure that no infection is present a colony treated in this way should be under observation for a considerable period after brood rearing has begun in it before it is used again.

Further reference to the technique used in these studies will be made as the experiments are recorded.

Fig. 8.—Capsule used in determining the thermal death point of spores.

## THERMAL DEATH POINT OF AMERICAN FOULBROOD SPORES

In an earlier paper (24) the results from preliminary experiments were given which indicate the amount of heating that is necessary to destroy the spores of American foulbrood when they are suspended in water. The importance of heat as a means for the destruction of these spores in practical apiculture is so great that a further development of the subject during these investigations seemed justifiable.

### RESISTANCE OF AMERICAN FOULBROOD SUSPENDED IN WATER TO HEATING

In preparing the spore material for heating a much diluted aqueous suspension of the disease material is drawn into capsules (fig. 8) made from glass tubing of small bore. After being sealed in a flame they are immersed in a water bath having a temperature and for the period desired in the heating.[1] Cultures then are made using a loopful of the suspension from the capsule and brood-filtrate-egg-yolk-suspension agar.

The results of the experiments given in the following table indicate the approximate amount of heating that is necessary for the destruction of the spores suspended in water.

TABLE I.—*Preliminary experiments indicating the thermal death point of the spores of Bacillus larvae suspended in water* [2]

FIRST SET OF EXPERIMENTS. DISEASE MATERIAL RECEIVED FROM AMERICA.

| Temperature. | | Period of heating. | Results as shown by cultures, October, 1913. |
|---|---|---|---|
| °C. | °F. | *Minutes.* | |
| 0 | 0 | 0 | Numerous spores alive (check). |
| 90 | 194 | 20 | Many spores not killed. |
| 94 | 201 | 10 | One spore not killed. |
| 96 | 205 | 10 | All spores killed. |
| 97 | 207 | 10 | Do. |
| 98 | 208 | 10 | Do. |
| 99 | 210 | 10 | Do. |
| 100 | 212 | 5 | Do. |
| 100 | 212 | 10 | Do. |

[1] About two minutes are allowed for the suspension within the capsule to reach the temperature of the water outside before time is reckoned.

[2] Fractions are omitted in this paper, the nearest whole number being given.

SECOND SET OF EXPERIMENTS. DISEASE MATERIAL RECEIVED FROM ENGLAND.

| Temperature. | | Period of heating. | Results as shown by cultures, October, 1913. |
|---|---|---|---|
| °C. | °F. | *Minutes.* | |
| 0 | 0 | 10 | Numerous spores alive (check). |
| 91 | 196 | 10 | Spores not killed, about $\frac{1}{10}$ as many as in check. |
| 95 | 203 | 10 | All but 2 spores killed. |
| 96 | 205 | 10 | All spores killed. |
| 98 | 208 | 10 | Do. |
| 99 | 210 | 10 | Do. |
| 100 | 212 | 1 | Spores not killed, about $\frac{1}{4}$ as many as in check. |
| 100 | 212 | 2 | All but 100 spores killed. |
| 100 | 212 | 3 | All but 20 spores killed. |
| 100 | 212 | 4 | Do. |
| 100 | 212 | 5 | Do. |

THIRD SET OF EXPERIMENTS. DISEASE MATERIAL RECEIVED FROM FRANCE.

| Temperature. | | Period of heating. | Results as shown by cultures, October, 1913. |
|---|---|---|---|
| °C. | °F. | *Minutes.* | |
| 0 | 0 | 10 | Numerous spores alive (check). |
| 90 | 194 | 10 | Spores not killed, almost as many as in check. |
| 92 | 198 | 10 | Spores not killed, about $\frac{1}{4}$ as many as in check. |
| 93 | 199 | 10 | All but 30 spores killed. |
| 94 | 201 | 10 | All but 100 spores killed. |
| 96 | 205 | 10 | All but 12 spores killed. |
| 98 | 208 | 10 | All but 1 spore killed. |
| 99 | 210 | 10 | All spores killed. |

FOURTH SET OF EXPERIMENTS. DISEASE MATERIAL RECEIVED FROM CUBA.

| Temperature. | | Period of heating. | Results as shown by cultures, January, 1915. |
|---|---|---|---|
| °C. | °F. | *Minutes.* | |
| 92 | 198 | 10 | Numerous spores not killed. |
| 93 | 199 | 10 | Do. |
| 94 | 201 | 10 | Fewer spores not killed. |
| 95 | 203 | 10 | Do. |
| 96 | 205 | 10 | Do. |
| 97 | 207 | 10 | Fewer alive than at 96° C. |
| 98 | 208 | 10 | Fewer alive than at 97° C. |
| 99 | 210 | 10 | About 12,000 spores not killed. |
| 100 | 212 | 10 | About 200 spores not killed. |
| 100 | 212 | 11 | Do. |
| 100 | 212 | 12 | Do. |

From Table I it will be observed that all the spores taken from the American sample were killed by heating at 96° C. for 10 minutes; that all of those from the English sample were also killed at 96° C. in 10 minutes; that all of those from the French sample were killed at 99° C. in 10 minutes; and that all of those from the Cuban sample were killed at 100° C. in 11 minutes. Spores from the different samples varied, therefore, in their resistance to heating, those from the American and English samples being slightly less resistant than those from the French one which showed in turn slightly less resistance than those from the Cuban sample.

By a comparison of the results it will be observed that many spores among those heated were destroyed at 90° C. in 10 minutes and that the percentage remaining alive when the higher temperatures were used rapidly decreased. The results indicate also that by increasing the temperature the time required to destroy the spores is decreased and vice versa.

Further studies relative to the thermal death point of the spores were made on disease samples received from different localities in the United States. The technique followed is similar to that given on page 22. The results obtained are summarized in Table II:

TABLE II.—*Variation in thermal death point of American foulbrood spores from different localities in the United States*[1]

| Temperature. | | Period of heating. | Source of samples. | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Wash. | Minn. | Nebr. | Ohio. | Ill. | Colo. | Wis. | Penn. 4529. | Penn. 4507. | Ohio 4519. | Mont. |
| °C. | °F. | *Minutes.* | | | | | | | | | | | |
| 100 | 212 | 10 | − | − | + | + | + | + | + | + | + | + | + |
| 100 | 212 | 5 | − | − | − | − | − | − | + | + | + | + | + |
| 100 | 212 | 1 | − | − | − | − | − | − | − | − | − | − | − |
| 98 | 208 | 10 | − | − | − | − | − | − | + | + | + | + | + |
| 95 | 203 | 10 | − | − | − | − | − | − | − | − | − | − | − |

[1] The minus sign in Tables II and IV indicates that the spores were not all killed and the plus sign that all of them were killed.

The results in Table II show that in none of the 11 cases were all of the spores killed in 1 minute at 100° C.; in 5 of the cases they were all killed in 5 minutes at this temperature while in 6 of them they were not. In two instances out of the 11 they were not killed in 10 minutes at 100° C. They were killed, however, at this temperature in 11 minutes. It will be seen also that in no instance were all of the spores killed at 95° C. in 10 minutes, but in 5 of the 11 cases all of them were killed at 98° C. within the 10 minutes.

By these results it is shown again that the spores of different samples do vary in their resistance to heat. No conclusion is drawn regarding the cause, environmental or otherwise, for this variation.

### TIME A FACTOR IN THE DESTRUCTION OF AMERICAN FOULBROOD SPORES BY HEAT

Suspensions of scales of American foulbrood in water were heated for different periods at 100° C. In the following table are recorded the results obtained when the spores from the Cuban sample were used:

TABLE III.—*Period of heating American foulbrood spores a factor when 100° C. is used*

| Temperature. | | Period of heating. | Results as shown by cultures, February, 1915. |
|---|---|---|---|
| °C. | °F. | *Minutes.* | |
| 100 | 212 | 0 | 25,000 spores present (estimated). |
| 100 | 212 | 1 | 4,000 spores not killed (estimated). |
| 100 | 212 | 5 | 148 spores not killed. |
| 100 | 212 | 6 | 220 spores not killed. |
| 100 | 212 | 7 | 248 spores not killed. |
| 100 | 212 | 8 | 44 spores not killed. |
| 100 | 212 | 9 | 7 spores not killed. |
| 100 | 212 | 10 | 14 spores not killed. |
| 100 | 212 | 11 | All spores killed. |
| 100 | 212 | 12 | Do. |
| 100 | 212 | 13 | Do. |
| 100 | 212 | 14 | Do. |
| 100 | 212 | 15 | Do. |

From Table III it will be noted that there were about 25,000 colonies in the check cultures. When a similar suspension was heated only about 4,000 spores remained alive after 1 minute at 100° C., 148 after 5 minutes, 44 after 8 minutes, 14 after 10 minutes, and none after 11 minutes. The conclusion is reached, therefore, that the time element is a factor in the destruction of the spores of American foulbrood by heat.

These results further show that the spores of different samples vary in their resistance to heat. It is interesting to note that by heating the disease material at 100° C. for one minute, more than 80 per cent of the spores were killed, and in 5 minutes, more than 99 per cent were destroyed.

In Table IV are summarized results which indicate further the value of the time element in the destruction of the spores by heat. In this instance spore material from 6 different localities was used, the heating being done at 95° C. with the spores suspended in water:

TABLE IV.—*Effect of the time element when 95° C. is used in heating American foulbrood spores*

| Temperature. | | Period of heating. | Source of samples. | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Cuba. | Colo. | Ohio 4504. | N. Y. | Ohio 4519. | Minn. |
| °C. | °F. | *Minutes.* | | | | | | |
| 95 | 203 | 12 | − | − | − | − | − | − |
| 95 | 203 | 15 | − | − | − | − | − | − |
| 95 | 203 | 20 | − | − | − | − | − | + |
| 95 | 203 | 40 | − | − | + | + | + | + |
| 95 | 203 | 50 | − | − | + | + | + | + |
| 95 | 203 | 60 | + | + | + | + | + | + |

From Table IV it will be observed that the spores from all of the six samples studied resisted in an aqueous suspension a temperature of 95° C. for 15 minutes. Spores from the Minnesota sample were destroyed in 20 minutes at 95° C., those from the Ohio and the New York samples in 40 minutes; and those from the Colorado and Cuban samples in 60 minutes. The time element is shown again to be an important factor in the destruction of American foulbrood spores.

These results show also, as was shown above, that the spores contained in different samples vary as to their thermal death point.

Other experiments were made showing the resistance of the spores of *Bacillus larvae* suspended in water to heat. At 93° C. (199° F.) spores from a Minnesota sample were destroyed in one hour, those from a Colorado sample in 1½ hours, while those from the resistant Cuban sample were not all killed in 3 hours. Experiments using Cuban, Colorado, and Pennsylvania samples showed in each instance that numerous spores remained viable after 2 hours' heating at 90° C. (194° F.).

### RESISTANCE OF AMERICAN FOULBROOD SPORES TO HEAT WHEN SUSPENDED IN HONEY

The effects of heating American foulbrood spores suspended in water are shown above. When suspended in honey a different degree of resistance is to be expected. The technique used to obtain facts relative to such resistance is quite similar to that described (p. 22) for the experiments in which the spores were heated in aqueous suspensions; instead of water, however, the spores were suspended in honey [1] or honey diluted with water. For testing whether or not the spores had been killed by the heating, cultures were used in some instances and bees in others.

In Table V are summarized some of the results obtained when the spores suspended in honey diluted with an equal volume of water were heated and tested by cultures:

TABLE V.—*American foulbrood spores heated in diluted honey* [1]

| Temperature. | | Period of heating. | Origin of sample. | Cultural results. April-May, 1915. |
|---|---|---|---|---|
| °C. | °F. | *Minutes.* | | |
| 98 | 208 | 10 | Nebraska | Numerous spores not killed. |
| 98 | 208 | 10 | New York | Do. |
| 98 | 208 | 20 | do | Do. |
| 98 | 208 | 20 | Nebraska | Do. |
| 98 | 208 | 20 | Illinois | Do. |
| 100 | 212 | 10 | New York | Do. |
| 100 | 212 | 20 | do | Do. |
| 101 | 214 | 8 | Washington State | Do. |
| 101 | 214 | 10 | Colorado | Do. |
| 102 | 216 | 8 | Cuba | Do. |
| 103 | 217 | 3 | Washington State | Do. |
| 104 | 219 | 3 | do | Do. |
| 105 | 221 | 3 | Cuba | About 500 spores not killed. |

[1] The altitude of the laboratory in Washington being nearly sea level the boiling temperature of water is almost 100° C. The higher temperatures recorded in this table and the following one were obtained by immersing the capsule containing the suspension in a solution of sodium chlorid and other salts.

Table V shows that numerous spores were alive after being heated in a suspension of honey diluted with equal parts of water for 20 minutes at 100° C. The spores of American foulbrood are not destroyed by heat as readily, therefore, when suspended in diluted honey as when suspended in water.

Experiments were made in which the spores were heated suspended in undiluted honey and tested by the cultural method. Table VI indicates the nature of the results obtained:

[1] The honey used was purchased at the market in sealed cans and was from the crop of the year preceding the experiments.

TABLE VI.—*American foulbrood spores heated in undiluted honey*

| Temperature. | | Period of heating. | Origin of sample. | Cultural results. April-May, 1915. |
|---|---|---|---|---|
| °C. | °F. | *Minutes.* | | |
| 100 | 212 | 10 | Cuba | Numerous spores not killed. |
| 100 | 212 | 10 | Washington State | Do. |
| 100 | 212 | 10 | Ohio | Do. |
| 100 | 212 | 20 | Cuba | Do. |
| 100 | 212 | 20 | Washington State | Do. |
| 100 | 212 | 20 | Ohio | Do. |
| 103 | 217 | 25 | Washington State | Do. |
| 105 | 221 | 20 | do | Do. |
| 105 | 221 | 20 | Cuba | Do. |
| 107 | 225 | 20 | do | Do. |
| 107 | 225 | 30 | do | Do. |
| 107 | 225 | 40 | do | Do. |

From the results given in Table VI it will be observed that in every instance the spores suspended in honey resisted 100° C. for more than 10 minutes. It will be noted that numerous spores were alive after being so heated for 20 minutes. In fact they resisted 105° C. for 20 minutes and more. It is shown that numerous spores from the resistant Cuban sample were still alive after 40 minutes heating at 107° C. When suspended in honey, therefore, spores are much more resistant to heat than when suspended in water (p. 22) or when suspended in diluted honey (p. 26).

While the thermal death point has not been definitely determined for the spores suspended in honey, the results obtained indicate that the point was being approached by the experiments of Table VI. It was found that the spores suspended in honey were killed readily by heating in the autoclave at 15 pounds pressure, being destroyed, in fact, by the time this pressure was reached. In making these tests the suspension in test tubes was brought to 100° C. before being placed in the autoclave. After the heating cultures were made.

### SPORES HEATED IN HONEY AND FED TO BEES

A further set of experiments was made in which American foulbrood material suspended in honey was heated to 100° C. and tested by the inoculation of colonies of bees. In performing the experiments a concentrated aqueous suspension of the disease material was added to hot honey, which is handled more readily than the cooler honey, until each 15 c.c. of honey suspension contained the material from 3 to 5 scales. This was then distributed in test tubes, each tube receiving about 15 c.c. of the suspension. These tubes were heated as were the aqueous suspensions in experiments reported above (p. 22), by immersing them in water. After heating, the contents of the tube were added to sirup and colonies were inoculated. In Table VII some of the experiments performed are summarized:

TABLE VII.—*American foulbrood spores heated in honey and fed to bees*

| Date of inoculation. | Temperature. | | Period of heating. | Results of inoculation. |
|---|---|---|---|---|
| 1916 | °C. | °F. | *Minutes.* | |
| Aug. 25 | 100 | 212 | 10 | American foulbrood produced. |
| Sept. 12 | 100 | 212 | 12 | No disease produced. |
| Do. | 100 | 212 | 15 | Do. |
| Do. | 100 | 212 | 18 | Do. |
| Aug. 25 | 100 | 212 | 20 | Do. |
| Sept. 12 | 100 | 212 | 25 | Do. |
| Aug. 11 | 100 | 212 | 30 | Do. |
| Aug. 25 | 100 | 212 | 30 | Do. |
| Do. | 100 | 212 | 45 | Do. |
| Oct. 11 | 100 | 212 | 90 | Do. |
| Do. | 100 | 212 | 120 | Do. |

It will be noted from Table VII that American foulbrood was produced after the scale material had been heated in honey for 10 minutes at 100° C., but was not produced when this temperature was maintained for 12 minutes or longer. The amount of heating required to prevent American foulbrood in the experiments recorded in this table is not as great as might have been expected from an examination of Table VI. The results tend to indicate that the virulence might have been affected somewhat before the spores were killed. Sufficient data to prove the point, however, are yet lacking.

From the various experiments relating to the thermal death point of American foulbrood spores recorded on the foregoing pages, it will be observed that 100° C. maintained for 10 minutes, with an exception now and then, is sufficient to kill all of the spores when suspended in water. It will be observed also that 98° C. applied for 10 minutes will do this in many instances and that 95° C. is sufficient in some cases. It is shown, furthermore, that a large majority of the spores are killed at 90° C. in 10 minutes and also that a very large number of them are killed at 100° C. in 1 minute.

That a difference exists in the thermal death point of American foulbrood spores was expected. A difference is seen in the spores from different samples, in those from different larvæ or pupæ in the same sample, and indeed in those of the same larvæ or pupæ. The maximum difference, when expressed in terms of degrees of temperature, between the most resistant spores of any two samples among those studied, the temperature being maintained for 10 minutes, is approximately 7° C.; and when expressed in time, the temperature being maintained at 100° C., is approximately 7 minutes. These differences are for the spores suspended in water. Suspended in honey a greater difference is to be expected. That still greater variations exist than those observed in these studies is most certain. To meet such differences the beekeeper has been urged to employ for the destruction of the spores in practical apiculture somewhat

more heat than the minimum amount that is required as determined by experimental studies.

## RESISTANCE OF AMERICAN FOULBROOD SPORES TO DRYING

That the American foulbrood spores are able to withstand much drying has long been realized by beekeepers. Scales of American foulbrood obtained in 1907 from colonies in which the disease had been produced through experimental inoculation were stored in the laboratory. Each succeeding year for 9 years tests were made relative to the viability of the spores in this material. In 1916 they were still alive and as resistant to heat and as virulent as at any previous time. It is most likely that they would have withstood the drying at room temperature for a very much longer period than the 9 years.

## RESISTANCE OF AMERICAN FOULBROOD SPORES TO DIRECT SUNLIGHT

### RESISTANCE OF AMERICAN FOULBROOD SPORES WHEN DRY TO DIRECT SUNLIGHT

In conducting experiments relative to the resistance possessed by spores of *Bacillus larvae*, when dry, to the direct rays of the sun, a heavy aqueous suspension is made of the scale material. This is spread in a thin film in Petri dishes and allowed to dry, the amount of disease material in each dish being equal to that in from 3 to 5 scales. The dry layer of disease material is then exposed to the direct rays of the sun and after different intervals of time cultures are made to determine whether the spores are viable. Table VIII gives a summary of the experiments made:

TABLE VIII.—*Resistance of spores of Bacillus larvae when dry to the sun's rays*

| Date of experiment. | Period of exposure | Results as shown from cultures. |
|---|---|---|
| 1916 | *Hours.* | |
| Aug. 31 | 2 | Numerous spores not killed. |
| Aug. 28 | 4 | Many spores not killed. |
| Sept. 5 | 5 | Do. |
| Do. | 7 | Several spores not killed. |
| Do. | 10 | Few spores not killed. |
| Sept. 6 | 11 | Do. |
| Sept. 28 | 12 | Many spores not killed. |
| Sept. 19 | 29 | Several spores not killed. |
| Sept. 25 | 38 | A dozen spores not killed. |
| Aug. 23 | 28 | All spores killed. |
| Aug. 28 | 37 | Do. |
| Sept. 23 | 41 | Do. |
| Sept. 25 | 41 | Do. |
| Sept. 16 | 44 | Do. |
| Do. | 61 | Do. |
| Sept. 25 | 79 | Do. |

From Table VIII it will be observed that all of the spores were killed by the direct rays of the sun in from 28 to 41 hours. It is natural to expect that the period required would depend upon the

date and time of exposure and somewhat upon the thickness of the film of disease material exposed. The climatic conditions during August and September, the time of the experiments, were naturally favorable for the destruction of bacteria by sunlight. The exposures were made only when the sky was clear, preference being given to the middle portion of the day.

#### REISISTANCE OF AMERICAN FOULBROOD SPORES WHEN SUSPENDED IN HONEY TO DIRECT SUNLIGHT

The technique used is as follows: A concentrated aqueous suspension of spore-containing material is added to honey in Petri dishes, each dish receiving the disease material equal to that of from 3 to 5 scales. These are exposed to the direct rays of the sun. The tops are used with the dishes to prevent robbing by bees. After different periods of time the contents of a single dish are added to sirup and fed to a colony free from disease. A set of experiments made is summarized in Table IX.

TABLE IX.—*Spores of American foulbrood in honey exposed to the sun*

| Date of experiment. | Period of exposure. | Results of inoculation. |
|---|---|---|
| 1916. | *Hours.* | |
| July 13 | 6 | Large amount of American foulbrood produced. |
| July 15 | 13 | Moderate amount of disease produced. |
| | *Weeks.* | |
| July 26 | 2 | Scattering cells of diseased brood. |
| Aug. 25 | 4 | Considerable diseased brood. |
| Sept. 1 | 5 | Do. |
| Aug. 10 | 4 | No disease produced. |
| Aug. 18 | 5 | Do. |
| Sept. 14 | 6 | Do. |
| Do. | 8 | Do. |

The data given in Table IX show that the spores of American foulbrood when suspended in honey were destroyed by the direct rays of the sun in from 4 to 6 weeks. As the suspension in each dish contained the disease material of from 3 to 5 scales it is evident from the results obtained that many of the spores must have been destroyed in a comparatively short period.

It will be readily appreciated that here again the period required for the destruction of the spores will vary greatly with the intensity of the sun's rays to which the honey suspension is subjected. In these preliminary experiments the period of exposure represents the entire time from the beginning of the exposure to the time of inoculation. There were days during the exposure on which the sun shone a great deal, others on which it shone very little, and still others on which it did not shine at all. The comparatively low temperature of the honey suspension attained during the exposure to the sun could not have been an important factor in the destruction of the spores.

## RESISTANCE OF AMERICAN FOULBROOD SPORES TO FERMENTATION

A few preliminary experiments relative to the effect of fermentative processes on the spores of American foulbrood were made. The processes involved concern chiefly the sugar splitting and the proteolytic enzymes. In experiments relative to the former, the scale material was suspended in a 20 per cent aqueous solution of honey and, in those relative to the latter, it was suspended in a 1 per cent aqueous solution of peptone. In each case a small bit of soil was added to inoculate the suspending solution still further. These suspensions, respectively, were distributed in test tubes and allowed to undergo fermentation. Observations were made at incubator and outdoor temperatures. The outdoor temperature was that which obtained in an empty hive body covered, and standing in the apiary at the time of the year shown by the dates of the experiments. After intervals reckoned in days colonies free from disease were inoculated, each with a suspension from a single tube. The experiments are summarized in Table X:

TABLE X.—*Resistance of the spores of Bacillus larvae to fermentation*

| Date of experiment. | Nature of the suspension. | Period. | Temperature. | Results of inoculations. |
|---|---|---|---|---|
| 1916. | | *Days.* | | |
| Aug. 14.. | Honey | 26 | Outdoors | American foulbrood produced. |
| Sept. 9... | do | 53 | do | Do. |
| Aug. 11.. | do | 24 | Incubator | Do. |
| Sept. 9.. | do | 53 | do | Do. |
| Aug. 14.. | Peptone | 26 | Outdoors | Do. |
| Sept. 9.. | do | 53 | do | Do. |
| Aug. 11.. | do | 24 | Incubator | Do. |
| Sept. ... | do | 53 | do | Do. |

From Table X it will be seen that in the presence of fermentative processes in the honey solution and in the peptone solution the spores were alive and virulent after 53 days at incubator and at outdoor temperatures. It is quite probable that they would have remained so for a very much longer period.

## RESISTANCE OF AMERICAN FOULBROOD SPORES TO CHEMICAL DISINFECTANTS

While a mass of data was obtained relative to the effect of chemical agents on the spores of American foulbrood, the results are still considered as being preliminary in nature. In conducting the experiments a suspension of disease material in an aqueous solution of the disinfectant was used. This was drawn into capsules [1] (fig. 9)

[1] The ampules used are made from glass tubing of small bore by heating and drawing it into three bulbs. The two intermediate constricted portions are wrapped with cotton. After being sterilized the ampules are filled with the spore-containing suspension sealed and placed at different temperatures. Incubator, room, and outdoor temperatures were employed. At the time of making the cultures the ampule is broken at the two intermediate constrictions and the spore-containing suspension used in making the inoculations is obtained from the intermediate bulb. By this method an immersion of the spores in the solution for the entire period is assured.

and sealed. After different intervals of time the suspension was cultured, using brood-filtrate-egg-yolk-suspension agar.

The results show that spores of American foulbrood were alive after being in suspension in 1, 2½, and 5 per cent aqueous solutions of carbolic acid (commercial) for months at room, outdoor, and ice-box temperatures, respectively. The maximum period during which any suspension was kept at ice-box temperature was 32 months. At incubator temperature the period during which the spores remain alive in 5 per cent carbolic acid is better reckoned in weeks than months. In 5 and 10 per cent solutions, respectively, of formalin (37 per cent formaldehyde) it was found that they were viable after 6 hours, and in a 20 per cent solution they were alive after 30 minutes. While the spores were alive in the formalin suspensions after these periods, the evidences obtained indicate that the chemical death point was being reached. Mercuric chlorid was resisted for days in 1 to 1000 and 1 to 500 solutions, respectively.

FIG. 9.—Capsule used in determining the resistance of spores to chemical disinfectants.

Inasmuch as the spores of American foulbrood resist the action of carbolic acid for more than a month under fairly favorable conditions for their destruction, this agent could scarcely be used with profit in practical apiculture. As the destruction of the spores with formalin is a question of hours only, it is possible in well-selected cases that use could be made of this agent. Since mercuric chlorid in strengths ordinarily used is resisted by the spores for days and furthermore is such a violent poison, its value as a disinfectant in American foulbrood is evidently limited. The general conclusion in regard to chemicals as disinfectants is, therefore, that they offer very little promise as a practical means for the destruction of the spores of American foulbrood.

## EFFECT OF DRUGS ON AMERICAN FOULBROOD

Divers experiences have been reported by beekeepers relative to the value of drugs in the treatment of the bee diseases. The fact that the spores of *Bacillus larvae* possess a marked resistance to chemical disinfectants tends to discourage hope that such substances would be of much therapeutic value when employed as drugs in American foulbrood. Such a conclusion, naturally, would not follow necessarily. Some data relative to the effects of drugs on this disease have been obtained through experimental inoculations using beta-naphthol, U. S. P.; carbolic acid (phenol), C. P.; oil of eucalyptus,

U. S. P.; formic acid, C. P.; salicylic acid, U. S. P.; salol (phenyl salicylate, U. S. P.); and quinin (bisulphate of quinin, U. S. P.).

In making the inoculations a suspension of scale material in water is added to medicated honey and colonies free from the disease are fed. The different drugs are used in different proportions. Honey, rather than sirup, is employed since the bees will take the drugs in higher proportions when in honey than when in sirup. The strength that they will take varies somewhat with the conditions present. The higher proportions recorded in Table XI summarizing the experiments approximate the maximum amount that can be employed:

TABLE XI.—*Indicating the effect of drugs on American foulbrood*

| Date of experiment. | Drugs. | Strength. | Results of inoculation. |
|---|---|---|---|
| 1916. | | | |
| July 11 | Betanaphthol | 1:2000 | American foulbrood produced. |
| May 29 | .....do | 1:1000 | |
| June 5 | .....do | 2:1000 | |
| July 11 | Carbolic acid | 1:2000 | |
| June 20 | .....do | 1:1000 | |
| Do | .....do | 2:1000 | |
| May 29 | .....do | 10:1000 | |
| Do | Oil of eucalyptus | 4:1000 | |
| July 11 | Formalin | 3:1000 | |
| Do | Salicylic acid | 1:2000 | |
| May 29 | .....do | 1:1000 | |
| June 5 | .....do | 2:1000 | |
| July 11 | Salol | 1:2000 | |
| May 29 | .....do | 1:1000 | |
| June 5 | .....do | 2:1000 | |
| July 11 | Quinin | 2:1000 | |
| May 29 | .....do | 3:1000 | |
| June 5 | .....do | 10:1000 | |

From Table XI it will be seen that American foulbrood was produced in all cases in which colonies were fed a suspension of diseased material in honey medicated with betanaphthol, carbolic acid, eucalyptus, formic acid, salicylic acid, salol, and quinin respectively in the proportions noted. In some of the experiments medicated sirup free from the spores was fed to the inoculated colony on a few successive days following the inoculation, and in some instances both preceding and following it. Whether these treatments with the medicated sirup produced any effect on the infection—positive or negative—was not determined definitely.

The results thus far obtained indicate that the drugs cited here can not be depended upon, for the present at least, in the treatment of American foulbrood. They do not preclude the possibility, however, that other drugs might be used with profit, but they do emphasize the fact that beekeepers should make sure that the value of a drug has been clearly demonstrated before it is used.

The results recorded on the foregoing pages relative to the resistance of American foulbrood spores to heat, drying, fermentation, sunlight, chemical disinfectants, and drugs, it will be observed, are only approximate from a strictly technical viewpoint. For practical purposes, however, they are in most instances entirely adequate. In using any of these results in devising means for the destruction of the spores in practical apiculture the time element determined by the experiments should be somewhat increased in each instance.

## MODES OF TRANSMISSION

Observations made during these studies point to certain paths by which American foulbrood is most likely to be transmitted. The evidences obtained tend to support certain views which have been entertained by beekeepers and to negative others which have been suspected by some as possible. Inasmuch as the disease may be produced in larvæ by feeding a colony *Bacillus larvae* in pure cultures, or from decaying remains of brood dead of the disease, it would seem that the portal of entry of the virus is somewhere along the alimentary tract of the larva. The fact leads at once to the suspicion that the food of bees contaminated with disease material is a very probable source of infection. Were the water supply likewise contaminated naturally it also would be a probable source of infection. Two prerequisites for the appearance of American foulbrood in a colony are (*a*) larvæ of the feeding age, and (*b*) a sufficient amount of disease material in the food or water supply of bees. A small amount of brood or a heavy flow of nectar, therefore, would tend to reduce the likelihood of colony infection under conditions otherwise favorable for infection.

Bees manifest a tendency to remove brood dead of American foulbrood as shown by the remains of partially removed larvæ and pupæ dead of the disease. The removal is done piecemeal and is accomplished more readily when the brood is recently dead than when the decaying remains are at all viscid or dry. Were the fate of the removed fragments definitely known much more could be said concerning the spread of the disease in the colony. It has been observed that a small amount of infection—a dead larva or pupa here and there—may be present in a colony for months, a year, and even longer, without causing a heavy infection in it. There is considerable evidence to support the belief that occasionally in cases of light infection the disease may disappear unaided by treatment. Such a phenomenon frequently takes place in sacbrood and indeed should be expected to occur now and then in American foulbrood. It should be emphasized that such a course for the disease, if it occurs at all, is unusual. Although American foulbrood

usually spreads more or less rapidly within an infected colony, the fact remains that it frequently does not.

It was observed that many diseased colonies could be present in the experimental apiary without causing the infection to be transmitted to other colonies in the apiary. This fact is naturally very significant. On account of it certain views concerning the manner of transmission of the disease, which might otherwise be regarded as probable, are rendered untenable. If flowers visited by bees from infected colonies, and later by bees from healthy ones, are a likely source of infection, or if the water supply is such a source, or if drones are a means by which the disease is likely to be transmitted, there would have been a different observation to report.

In a few instances in the experimental apiary colonies near heavily infected ones became infected. That the disease may have been transmitted through the drifting or accidental straying of bees is one of the possible explanations for the disease in these cases. That a slow form of robbing might have taken place should be considered also as a possible explanation in these cases.

Brood comb containing brood dead of American foulbrood will transmit the disease when placed in a hive containing a healthy colony. The likelihood that the disease will be transmitted by combs from diseased colonies, which contain honey but no brood, probably is frequently overestimated. It would seem that a spread of the disease in this way would depend considerably upon the amount of infection that was present in the colony from which the combs were removed. This would depend also somewhat upon the presence or absence of brood in the colony to which the combs were given. Sufficient facts are wanting to make definite statements in regard to the probability of infection in such cases. Robbing material containing the spores of American foulbrood from any source is likely to transmit the disease although it does not necessarily do so.

How often the disease would be transmitted through the medium of hives which have housed infected colonies, if used without flaming, has not been definitely determined. Experimental colonies have been placed in such hives and kept in them for a year with the result that no disease appeared.[1] It probably will be found that in many cases the treatment of the hive bodies is not necessary to insure that the disease will not be transmitted by them. From the results obtained by practical apiarists and by observation made in the experimental apiary during these studies the fact has been well established,

[1] An experiment in which four colonies were used, was made as follows: The insides of the top and bottom board of each of two hive bodies were washed with an aqueous suspension of spore-containing material and allowed to dry, and the inside of the walls of each of two others were similarly washed and allowed to dry. Colonies were transferred to these four hives during the summer. American foulbrood appeared in the two colonies housed in hives of which the tops and bottoms had been contaminated with disease material but did not appear in the other two. These results, naturally, are not conclusive but they are suggestive.

however, that the danger of infection from such hives is entirely removed by carefully flaming them inside. The destruction of the hive is, therefore, never necessary. Chemical disinfectants should not be relied upon for the destruction of the spores of American foulbrood as the spores possess a marked resistance to these agents. If any chemical is used, however, strong solutions of formaldehyde would seem to offer some promise of being efficient.

After disease material is removed from the hive by bees and is released from them the chances that a bee will come in contact with the contained spores again is comparatively slight. Furthermore such spores must withstand certain agencies in nature which tend to destroy them, thus decreasing the chances still further that disease will result from them. If exposed to the direct rays of the sun the spores will be destroyed more or less readily (p. 29). Much less is to be expected from fermentation (p. 31) or drying (p. 29). Should the spores reach the soil or running water the likelihood that bees will come in contact with them and carry them to the feeding brood is indeed slight. Should the spores reach the water supply of the bees and should this supply be a stagnant or slow-moving body of water, the chances naturally would be somewhat increased.

Experiments in which queens taken from diseased colonies were introduced into healthy ones were not kept under observation for a sufficiently long period to justify a definite conclusion as to the probability of the disease being transmitted by such queens, but the data secured indicate strongly that the danger of transmission in this way has been overestimated at times. The favorable results obtained by beekeepers using the shaking treatment should tend to allay fear in regard to the transmission of the disease by this route. Indeed the facts thus far obtained suggest that the transmission of the disease by way of the queen should not be expected.

The spread of the disease by means of the clothing or hands of the apiarist is not to be feared especially. The hive tool, if brought in direct contact with dead larvæ in testing for the presence of disease, might serve to transmit infection, but during the usual manipulations it would not. The practice sometimes followed by beekeepers of thrusting the hive tool into the soil to clean it would seem to be a safe procedure. Washing the hands with water instead of disinfectants is more convenient and is sufficient. Reasonable care should be taken, of course, that the water used does not become or reach the water supply of bees.

## DIAGNOSIS

American foulbrood is the easiest of the brood diseases to diagnose. Methods by which this can be done in the laboratory are given in an earlier publication (16). The diagnosis as a rule can be

made from the symptoms (p. 5) alone. No helpful sign is found in the appearance of the adult bees. A weak colony justifies a suspicion that a disease is present. In American foulbrood, as in European foulbrood and sacbrood, a sample suitable for diagnosis must contain the remains of brood dead of the disease.

The colony symptoms which are usually adequate for a definite diagnosis of American foulbrood are the following: The death of larvæ in capped cells after the endwise position has been assumed (Pls. I, II, III, VI), and the death of pupæ soon after transformation (Pls. IV, V, VI), the brown shade of dead brood, the viscidity (ropiness) of the decaying remains, the character of the scales, and the foulbrood odor.

#### BACTERIOLOGICAL EXAMINATION

A conclusive diagnosis of American foulbrood can always be made from a bacteriological examination of brood dead of the disease. With gross characters suggesting the disease the experienced can frequently make the tentative diagnosis definite by a water mount made from the decaying brood remains, or the scales. A very large number of spores free from rods with no other bacterial species present is the characteristic microscopic picture in the disease. The spores are those of *Bacillus larvae* (fig. 4; Pl. VII, B, D). As a rule, a stained preparation does not furnish much additional aid. In all cases of doubt cultures (agar plates are satisfactory) should be made. The absence of growth on the plates when the other data strongly suggest American foulbrood is sufficient for a positive diagnosis. In routine work the cultures should always be made. Occasionally further evidence that the spores present are those of *B. larvae* is desired. This can be obtained by the employment of methods and media given in this paper (p. 17).

#### DIFFERENTIAL DIAGNOSIS

##### EUROPEAN FOULBROOD

European foulbrood can be recognized (27) in most instances by the death of brood in uncapped cells, the yellow hue of larvæ recently dead changing later often to a brown, and an absence of the foulbrood odor. In cases in which these younger larvæ have been removed, the diagnosis of European foulbrood can very often be made from the remains of brood which has died in capped cells. In these cases the remains of dead larvæ and not of pupæ are encountered. These decaying masses pass through a stage at which they are somewhat viscid. The scales are rubberlike [1] in consistency. In these masses and in the scales large numbers of *B. alvei* are found.

[1] The term "rubberlike" needs interpretation. When applied to the scales of European foulbrood it means simply that they are less brittle than either those of American foulbrood or sacbrood. The property "elasticity" as usually thought of in common parlance in connection with rubber is not meant.

#### SACBROOD

Sacbrood is recognized (25) by the death of larvæ in capped cells and not of pupæ, by the saclike appearance of the dead remains, and by the absence of viscidity. The absence of microorganisms in the remains characterizes the microscopic picture.

#### OTHER CONDITIONS

Other brood conditions referred to as chilled brood, overheated brood, starved brood, and in some cases drone brood, must be differentiated from American foulbrood. The history of the case, the age of the brood at the time of death, and the absence of ropiness and of the foulbrood odor, usually make the diagnosis comparatively easy.

In some of the disorders of adult bees excrement of the adult bees is sometimes found in the cells of the brood combs. When dry the masses resemble somewhat brood-disease scales. The character of the masses together with the microscopic picture is usually sufficient for a diagnosis. In these cases a stained preparation is preferable. The fecal matter contains bacterial rods in large numbers. In all of these conditions the absence of *B. larvae* furnishes definite evidence that American foulbrood is not present.

### PROGNOSIS

Without treatment the prognosis in American foulbrood is decidedly grave, the rule being that the colony sooner or later dies as a result of the disease. This is true for experimental colonies and is true also, as has been proved by the experiences of beekeepers, for colonies in which the disease has occurred through natural means.

A colony heavily inoculated experimentally is not destroyed by disease in one month or two months. Loss in strength may be apparent, however, after one month. If a colony is inoculated early in the season and is heavily infected, it may die by midsummer; if less heavily infected, it may live longer but die later in the season. The infection may be so slight, indeed, that the colony may not be destroyed directly but be so weakened that it will die during the winter or surviving emerge a weakened colony in the spring and then die of the disease during the following bee season. When a colony during the summer contains much dead brood and becomes very weak as a result of the disease, it usually absconds. The queen is to be found among the bees to the last, and stores are not wanting as a rule.

A question of considerable interest but one which has not yet been completely answered is: Does an American foulbrood colony ever recover without treatment? Some beekeepers have entertained the

belief that occasionally it does. Experimental evidence indicates that it probably does. Colonies in the experimental apiary, with a larva or pupa dead of the disease here and there only, certainly did not become badly diseased for more than a year and apparently recovered. To have proved conclusively, however, that such colonies were completely free from possible infection from within would have necessitated observations over a much longer period.

It is known that worker, drone, and queen larvæ die of the disease. Theoretically it is possible that a colony might become queenless as a direct result of American foulbrood but care must be taken in attributing this condition to the disease, for very often queens are reared in diseased colonies, and to all appearances are healthy. Whether a larva once infected ever recovers from the disease is not known.

From the facts at hand it may be concluded, therefore, that the prognosis in American foulbrood in an untreated colony is especially grave. From the practical viewpoint, at least, complete recovery from the disease without treatment, if it occur at all, should be considered for the present to be the exception. While an infected colony may live for a long time and yield a profitable surplus for a considerable period, this is not the rule; more often the course of the disease is comparatively short, and the destruction of the colony the outcome.

## SUMMARY AND CONCLUSIONS

A brief summary of facts known about American foulbrood together with a few conclusions drawn from them are given here. Some of the facts have been known for many years, others are of more recent origin, and still others are new. All of them are supported by experimental studies recorded in the present paper.

1. American foulbrood is an infectious disease of the brood of bees caused by *Bacillus larvae*.

2. All larvæ—worker, drone, and queen—are susceptible to the infection; adult bees are not.

3. Man evidently is not susceptible to infection with the organism nor are the experimental animals.

4. So far the disease has not been encountered or produced in other insects than honeybees.

5. The brood of bees can be infected through feeding the spores of the bacillus to a colony.

6. The spores contained in a single scale are more than enough to produce considerable disease in the colony.

7. The portal of entry of the infecting agent is somewhere along the alimentary tract of the larva, most likely the stomach (mid-intestine).

8. Practically speaking there are no secondary invaders either during the life of the infected larva or during the decay of the remains.

9. The incubation period is approximately 7 days.

10. The brood is susceptible to infection at all seasons of the year.

11. More brood dies of the disease during the second half of the brood-rearing season than during the first half.

12. The disease is present at least in Australia, New Zealand, Denmark, England, Ireland, Germany, France, Switzerland, Canada, Cuba, and the United States. The ropy foulbrood of all these countries is one and the same disease.

13. Occurring as it does in such a wide range of climatic conditions, it is evident that the presence of the disease can not be attributed alone to any particular climate.

14. The course of the disease in the colony is not affected greatly, if at all, by the quality of food used by the bees, or by the quantity present.

15. Colonies in which the disease has been produced through artificial inoculation can be kept in the experimental apiary without transmitting the disease to others. This fact is of special importance not only in the technique of making studies, but also in the control of the malady.

16. The spores of American foulbrood remain alive and virulent for years in the dry remains (scales) of larvæ and pupæ dead of the disease and in cultures that have become and remain dry.

17. The spores are very resistant to most destructive agencies. A variation in resistance is noted both as to the individual spores of a sample and as to the spores contained in different samples.

18. Many of the spores are killed within 1 minute at 100° C. and all of them from some samples are killed in less than 5 minutes. In some instances 96° C. maintained for 10 minutes will destroy all of the spores while 98° C. will often do it. The most resistant of the spores studied when suspended in water have not withstood 100° C. for 11 minutes.

19. The spores withstand more heating when they are suspended in honey or honey diluted with water than when suspended in water.

20. The spores suspended in honey or diluted honey can be destroyed by 100° C. but it may require half an hour or more to do so.

21. American foulbrood spores when dry were destroyed by the direct rays of the sun in from 28 to 41 hours.

22. The spores when suspended in honey and exposed to the direct rays of the sun were destroyed in from 4 to 6 weeks.

23. The spores when suspended in honey and shielded from direct sunlight remained alive and virulent for more than a year. It

is very likely that they are capable of remaining so for a very much longer period.

24. The spores resisted the destructive effects of fermentation for more than 7 weeks at incubator and outdoor temperatures, respectively, and probably are able to withstand these agencies for a very much longer period.

25. The spores resist carbolic acid at room temperature in strengths ordinarily used as a disinfectant for periods of months; 1 to 1000 mercuric chlorid for days; 10 per cent formalin for hours.

26. Experiments recorded in the present paper indicate that drugs do not materially affect the course of the disease.

27. American foulbrood infection is transmitted primarily through the food of bees; possibly at times to some extent through their water supply. Robbing from the diseased colonies of the apiary, or from neighboring apiaries, is the most likely mode by which the disease is transmitted in nature.

28. The placing of brood combs containing diseased brood with healthy colonies will result in the transmission of the disease.

29. Flowers should not be considered as a likely medium through which infection may take place.

30. Whether the disease is ever transmitted by queens or drones has not been determined. That they have been overestimated at times as possible sources of infection seems likely.

31. It is quite probable that in many cases hives which have housed colonies infected with American foulbrood will not transmit the disease to healthy colonies transferred to them. Results from the present studies confirm the observation made by beekeepers that danger from this source may be removed by properly flaming such hives inside.

32. The clothing of those about an apiary, and the hands of the apiarist are not fruitful sources for the transmission of the disease.

33. Tools and bee supplies generally about an infected apiary will not transmit the infection in the absence of robbing from those sources.

34. American foulbrood usually can be diagnosed from the symptoms alone. A definite diagnosis can always be made from suitable samples by bacteriological methods.

35. The prognosis in the disease in the absence of treatment is decidedly grave but with proper treatment it is favorable.

36. From the technical viewpoint many of the problems considered in these studies have been solved only partially; from the practical point of view, however, the results are sufficient to make a logical, efficient, and economic treatment of American foulbrood possible.

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## EXPLANATION OF PLATES

### PLATE I

Brood affected with American foulbrood:

A.—American foulbrood produced by the feeding of pure cultures of *Bacillus larvae*, showing the later stages of decay of the brood.

B.—American foulbrood produced experimentally, showing irregular distribution of capped and uncapped cells, punctured caps, and scales.

C.—American foulbrood, showing sunken and punctured caps. The sample was shipped by mail for diagnosis.

### PLATE II

Healthy bee larvæ (prepupæ) and first and second postmortem stages of American foulbrood larvæ as described in the present paper:

A.—Cap of cell containing a healthy larva. Being recently constructed, it is convex.

B.—Cap of cell containing a larva recently dead of the disease. It is not unlike many of the caps over healthy larvæ.

C.—Cap of cell containing a diseased larva. It is slightly sunken.

D.—End view of a healthy larva. The cap was removed artificially. (In the laboratory this is done conveniently with fine pointed curved forceps or a dissecting needle.)

E.—End view of larva illustrating the earliest (first) stage of American foulbrood.

F.—End view of larva which has reached the second stage.

G.—Ventral view of a healthy larva of the age at which death from American foulbrood frequently occurs.

H.—Ventral view of larva illustrating the first stage after death.

I.—Ventral view of larva showing second stage.

### PLATE III

Third, fourth, and fifth stages of decay in American foulbrood larvæ:

A.—Punctured cap of cell containing a dead larva.

B.—End view of larva illustrating the fourth stage of decay. The cap was removed artificially as indicated by the torn edges.

C.—End view of larva illustrating the fifth stage of decay, the scale. The cap had been removed by the bees, as shown by the smooth condition of the mouth of the cell.

D.—End view of larva, third stage.

E.—End view of larva, fourth stage.

F.—End view of larva, fifth stage—the scale.

G.—Ventral view of larva, third stage.

H.—Ventral view of larva, fourth stage.

I.—Ventral view of larva, fifth stage—the scale.

### PLATE IV

Healthy pupæ and first and second postmortem stages of American foulbrood pupæ as described in the present paper:

A.—Healthy pupa of the age at which death from American foulbrood occurs.

B.—End view of pupa, first stage of the disease.

C.—End view of pupa, second stage.

D.—Ventral view of pupa of the age at which death from American foulbrood occurs.

E.—Ventral view of pupa, first stage.

F.—Ventral view of pupa, second stage.

### PLATE V

Third, fourth, and fifth postmortem stages of American foulbrood as described in the present paper:

A.—End view of pupa, third stage.

B.—End view of pupa, fourth stage.

C.—End view of pupa, fifth stage—the scale.

D.—Ventral view of pupa, third stage.

E.—Ventral view of pupa, fourth stage.

F.—Ventral view of pupa, fifth stage—the scale.

## PLATE VI

Various phases of the appearance of brood dead of American foulbrood:

A.—Larva in cell not yet capped, dead of American foulbrood.

B.—A developing bee dead of American foulbrood immediately before entering the pupal stage.

C.—A punctured cap with two holes.

D.—Larva dead of American foulbrood gnawed and partially removed by the adult bees.

E.—End view of larval scale. The cap was removed artificially.

F.—End view of pupal scale.

G.—Lateral view of healthy larva in capped cell.

H.—Lateral view of larval scale in the cell cut lengthwise. The cap had been removed by the adult bees.

I.—Lateral view of pupal scale in position in cell cut lengthwise.

## PLATE VII

Vegetative form, spore formation, spores, flagella, and giant whips of *Bacillus larvae*. Photomicrographs:

A.—Vegetative form from a 24-hour culture on the surface of brood-filtrate agar. × 1000.

B.—Spore formation and spores from the surface of brood-filtrate agar. × 800.

C.—Flagella. Stained by Loeffler's method as modified by Johnson and Mack. × 1000. (Retouched.)

D.—Spores from a stained smear made directly from a decaying larva dead of American foulbrood. × 1000.

E.—The production within the rods of separate elements, each being supplied with flagella. × 1000.

F.—Giant whips and small spherical bodies in the older cultures of *B. larvae* obtained from the water of condensation of a brood-filtrate-agar slant culture. Fixed in 50 per cent formalin and stained with carbol fuchsin. × 1000.

G.—Small and larger giant whips, and spherical bodies similar to those of F. × 1000.

A similarity existing between the microscopic appearance of giant whips and spirochetes caused Maassen (14) to believe them to be spirochetes and to introduce the name *Spirochaeta apis*.

H.—Very large whip, and spherical bodies similar to those of F and G. × 1000.

## PLATE VIII

Colonies and cultures of *Bacillus larvae:*

A.—Surface colony and deep colonies as seen in brood-filtrate agar plates.

B.—Surface colony on brood-filtrate agar more highly magnified.

C.—Deep colony in the same medium, magnified.

D.—Surface growth on brood-filtrate-agar slant.

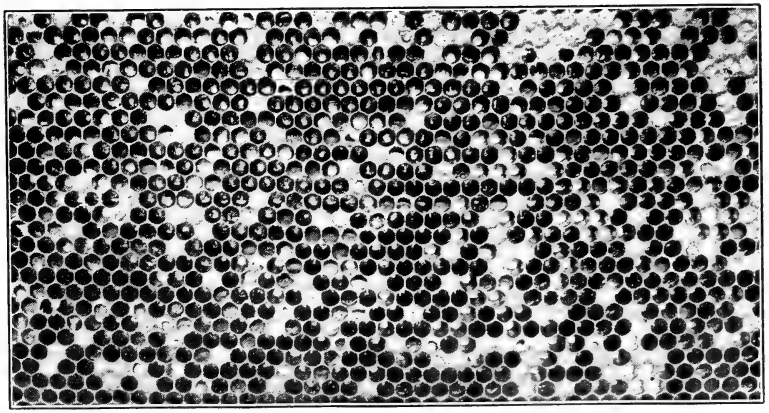
E.—Culture of *Bacillus larvae* in bee-larvæ agar when the Liborius method is used. The growth is throughout the upper third of the medium.

F.—The culture is similar to E but the growth is nearer the surface.

G.—The culture is restricted in the agar and is near to but not at the surface of the medium.

H.—The growth is less restricted in area and farther from the surface than in G.

I.—The growth is very restricted in area and is below the middle of the medium.



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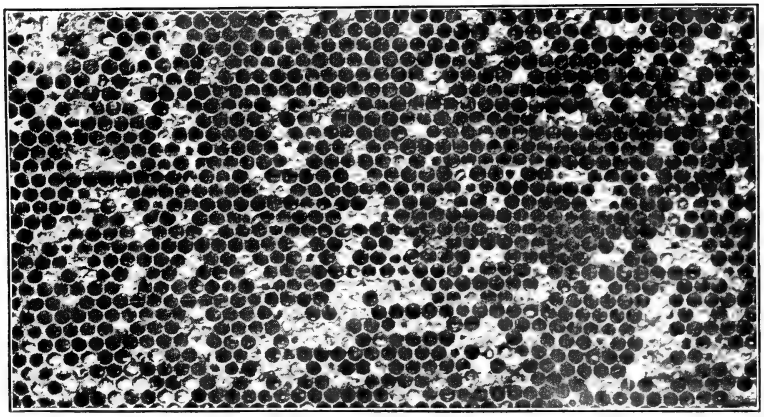

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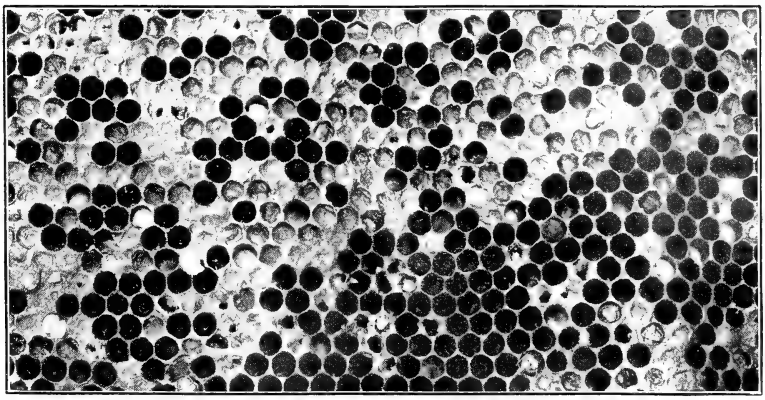
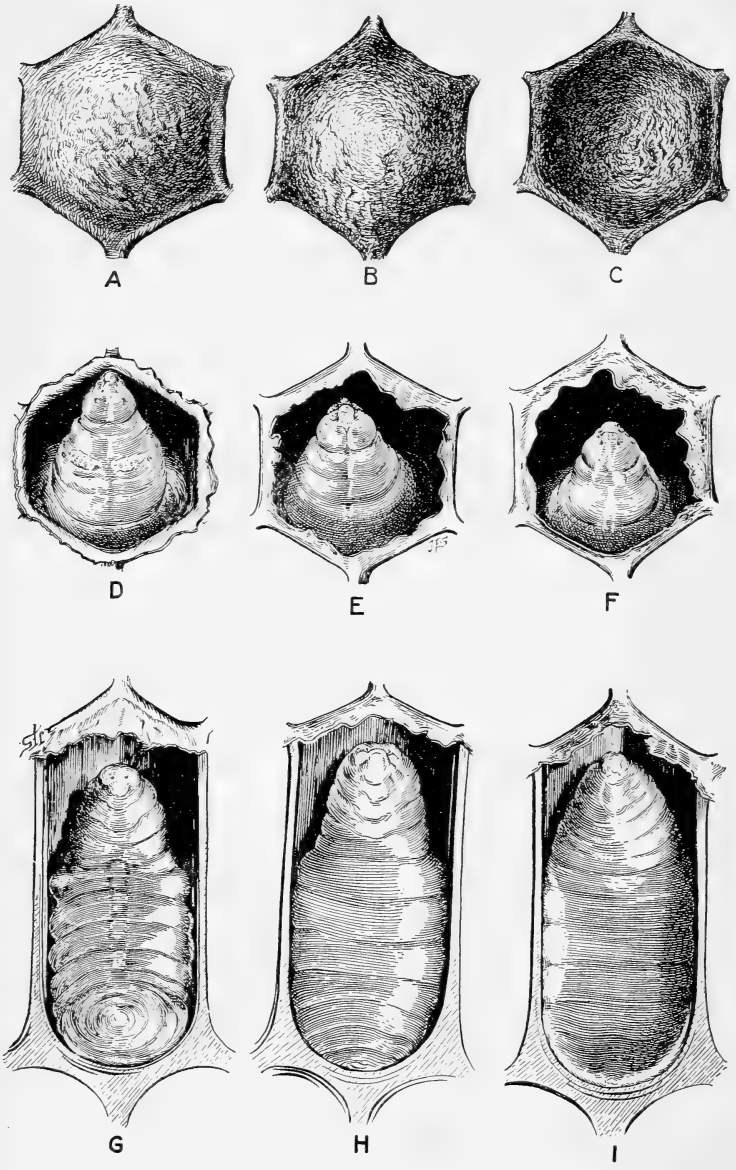


C

AMERICAN FOULBROOD.

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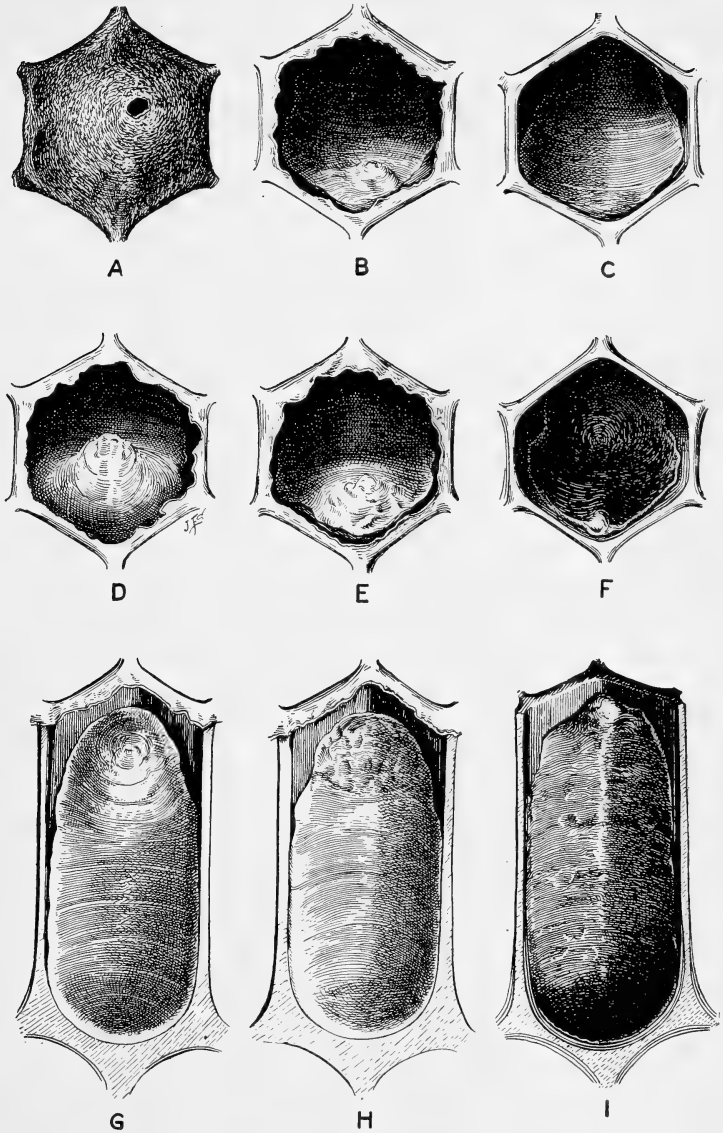


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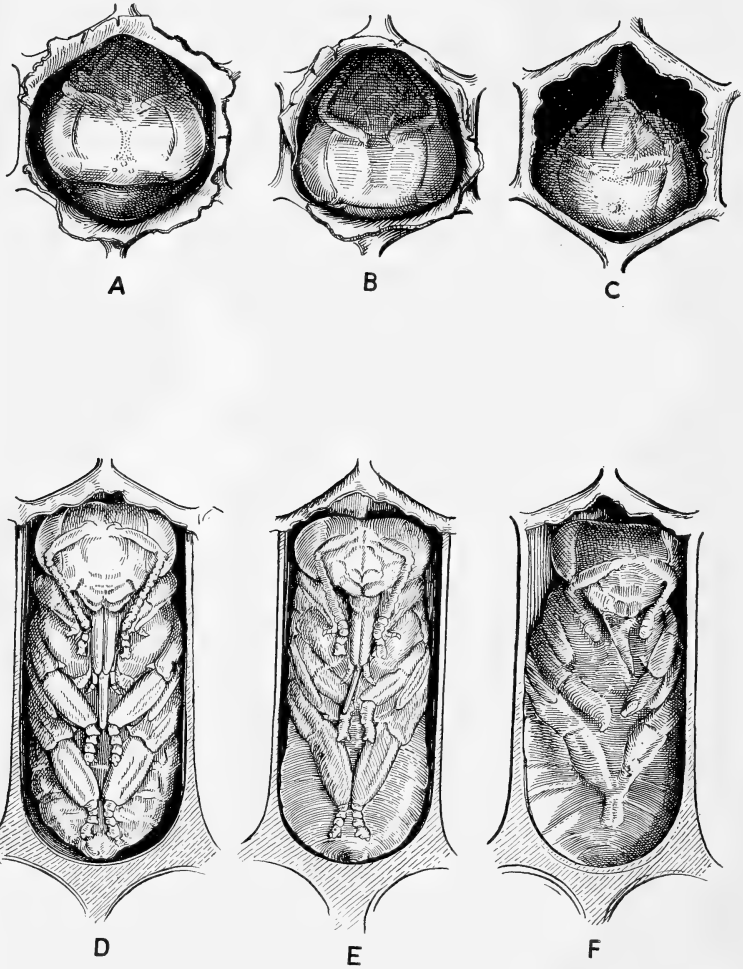


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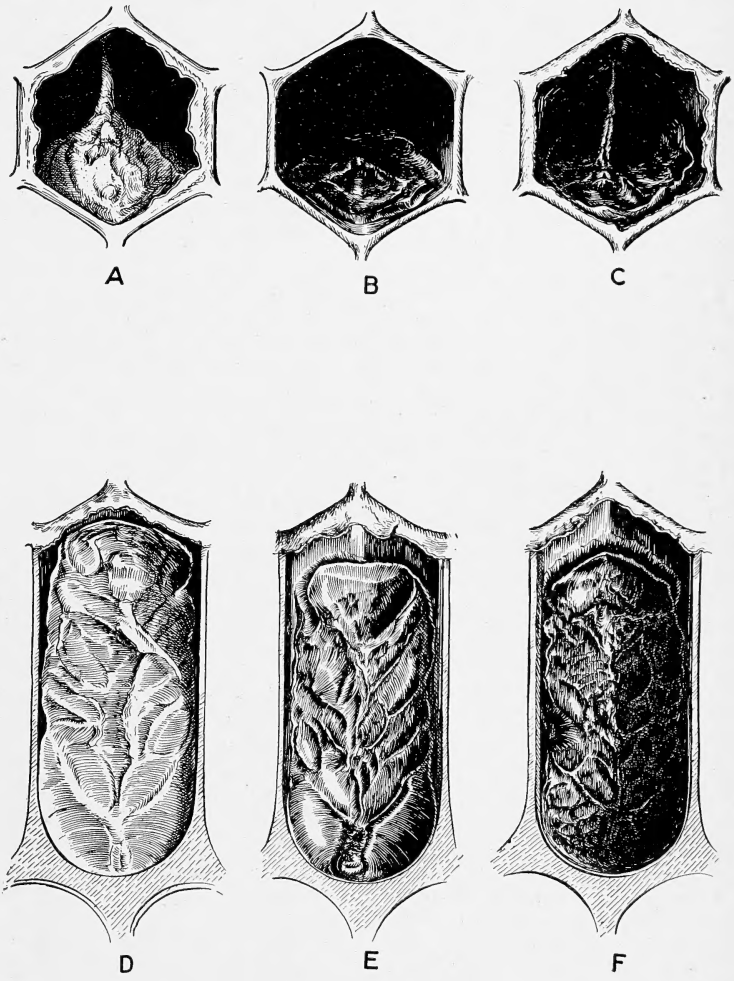


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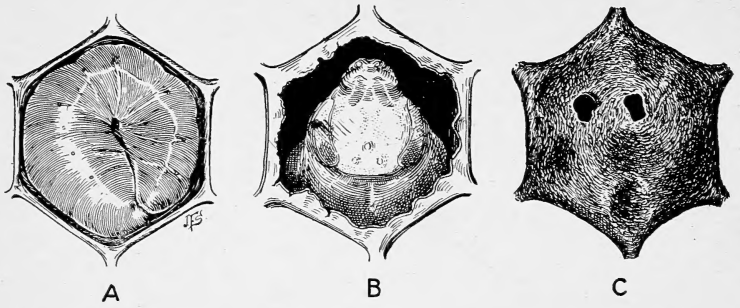


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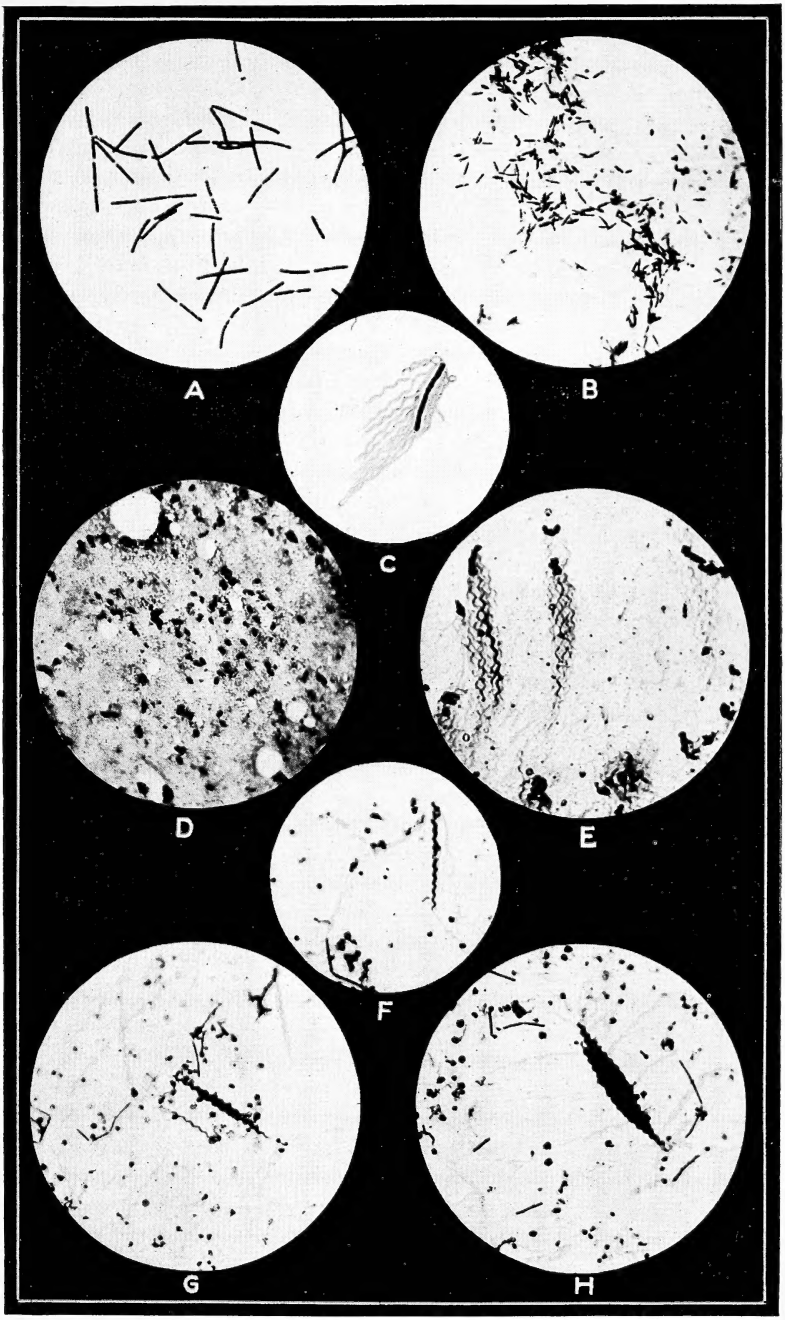


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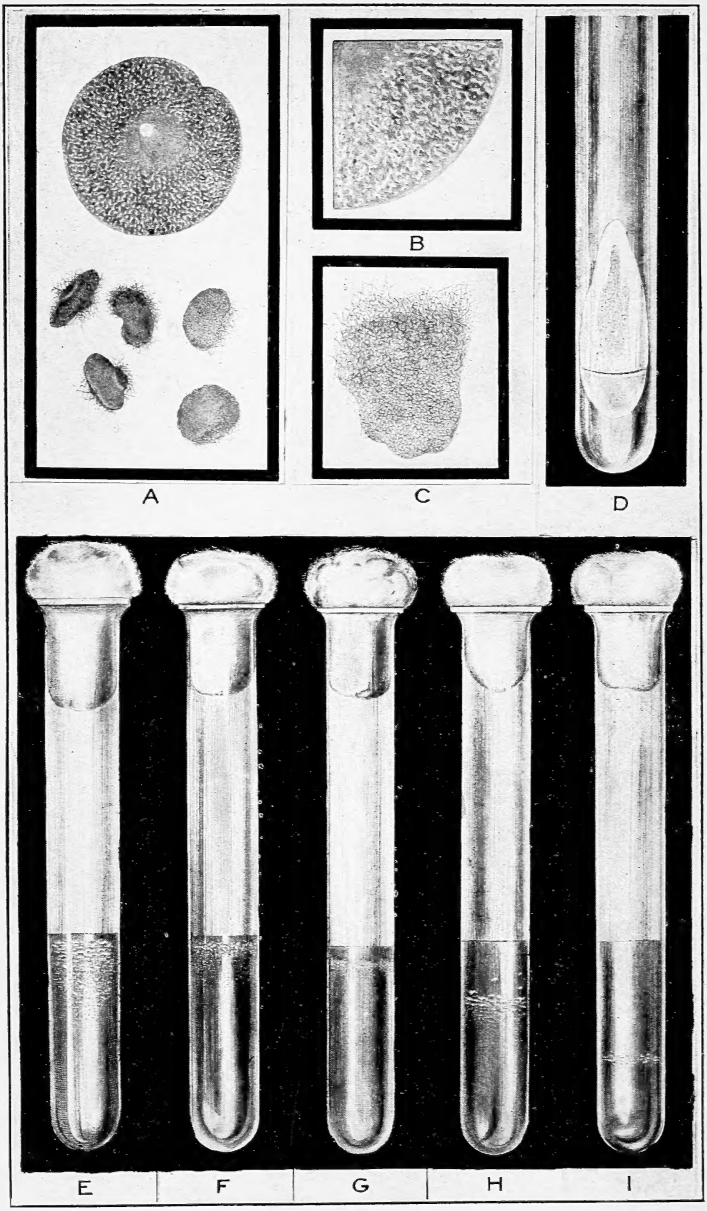


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